UNIVERSITY OF NAPLES "FEDERICO II"

DEPARTMENT OF AGRICULTURAL SCIENCES

AND

AN-NAJAH NATIONAL UNIVERSITY

FACULTY OF GRADUATE STUDIES

MASTER DEGREES IN

FOOD SCIENCE AND TECHNOLOGY

AND

NUTRITION AND FOOD TECHNOLOGY

EFFECT OF THE MEDITERRANEAN DIET ON THE GUT MICROBIOTA OF PREGNANT WOMEN: A CASE-CONTROL STUDY

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Co- Tutor:

Dr. Francesca De Filippis

Academic year 2020-2021

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Experimental thesis

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Academic year 2020-2021

Dedication

I dedicate my dissertation work to my family and many friends. A special feeling of gratitude to my loving parents, whose words of encouragement and push for tenacity ring in my ears. My sister Aziza and Inas have never left my side and are very special.

I also dedicate this dissertation to my many friends who have supported me throughout the process. I will always appreciate all they have done.

I dedicate this work and give special thanks to my wonderful mother and my best sisters for being there for me throughout the entire program. Both of you have been my best cheerleaders.

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الاقرار

أنا الموقعة أدناه، مقدّمة الرسالة التي تحمل العنوان:

EFFECT OF THE MEDITERRANEAN DIET ON THE GUT MICROBIOTA OF PREGNANT WOMEN:

A CASE-CONTROL STUDY

أقر بأن ما اشتملت عليه هذه الأطروحة إنما هو نتاج جهدي الخاص، باستثناء ما تمت الاشارة إليه حيثما ورد. وأن هذه الرسالة كاملة، أو اي جزء منها لم يقدم من قبل لنيل اي درجة او لقب علمي او بحثي لدى أي مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's Name: **اسم الطالبة:**

Signature: **التوقيع:**

Date: **التاريخ:**

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EFFECT OF THE MEDITERRANEAN DIET ON THE GUT MICROBIOTA OF PREGNANT WOMEN: A CASE-CONTROL STUDY

By
Yasmeen Adwan Matr
Supervisors
Mohammad Altamimi
Francesca De Filippis

Abstract

Aim:

The main aim of this study is to evaluate the possible influence of the adherence to the Mediterranean diet on gut microbiome composition during pregnancy.

Design:

Seventy-six healthy pregnant women participated in a parallel 9-months randomized controlled trial. Thirty-five participants were instructed to consume a diet inspired to Mediterranean dietary pattern (MedD), and 41 maintained their habitual diets (ConD). Dietary adherence and gut microbiome were monitored over the study period.

Design and results:

We retrieved dietary information and assessed gut microbiome by shotgun metagenomics in 76 pregnant women following Med and control diets. The majority of the women in MedD group increased the adherence to the MedD. We detected associations between the consumption of MedD and the abundance of beneficial microbes, such as some fiber-degrading

bacteria and some taxa associated with the degradation of polyphenols, whose role in the human gut warrants further research.

Conclusions:

Switching subjects to a MedD (increasing their adherence to the MedD) during pregnancy can be considered as a promising strategy to improve their gut microbiome, possibly influencing the gut microbiome development in newborns.

Chapter One
introduction

1.1 Gut microbiota

With the term “microbiota” we refers to a massive group of organisms (consist of all living members) that colonize specific sites (such as the human gut), including bacteria and other organisms such as protozoans, archaebacteria, viruses, and fungi. The gut microbiota plays an essential role in mucosal immunity, epithelial cell development, and metabolic activity in the gastrointestinal tract (Berg et al., 2020; Jandhyala, 2015; McKnite et al., 2012).

1.2 Composition and function of the gut microbiota

The main phyla present in the human gut microbiota are Firmicutes and Bacteroidetes, which form more than 90% of taxonomic composition, and are divided into more than 100 distinct bacterial families and genera. The composition of the gut microbiota appears dynamic during human life; rapid changes occur from birth till 3 years of age, after that greater constancy is achieved. However, later in life (after 60-65 years), appears acceleration in the tendency of irregular changes in gut microbiota (Grosicki et al., 2018).

Bacteria are found everywhere in the environment, water, air, and soil; in addition, they are present on human skin in large quantities, and of course in the gastrointestinal system. Bacteria have a variety of shapes, including

spherical, buds, rod, or spirillum shapes. Based on their tolerance to oxygen are classified into anaerobic, microaerophilic, or aerobic organisms. Depending on nutritional characteristics are classified as oligotrophic, phototrophic, or heterotrophic (Dieterich et al., 2018).

The number of bacterial cells raise steadily along the gastrointestinal tract, with a low amount in the stomach; but a very high number in the large intestine (colon). The stomach and small intestine (the proximal duodenum) have a harsh environment, so a small number of bacteria can survive and tolerate the very low pH (acidic environment), bile, and pancreatic enzymes (Dieterich et al., 2018).

Many studies reported that a high load of bacteria and changes in the gut microbiota composition are characterized in a healthy pregnancy. The gut microbiota composition during the first trimester of pregnancy is the same as that of non-pregnant females. However, the microbial composition of the gut microbiota dramatically changes at each trimester of pregnancy. These alterations are distinguished by the increased abundance of phyla Proteobacteria and Actinobacteria; and a decrease in alpha (α) diversity (reduction of diversity of taxa within a single subject) (Nuriel-Ohayon et al., 2016; Ferrocino et al., 2018).

1.2.1 Stomach

Research on stomach microbiota neglected for years because the concept of "stomach as a sterile organ" that does not host bacteria due to its acid production. In addition, gastric bile acid reflux, mucosal layer thickening, and efficacy of gastric peristalsis may impair colonization of the stomach. Moreover, nitrates found in food and saliva are degraded by the lactobacilli in the mouth to nitrite and, once in the stomach, are transformed by gastric juice into nitric oxide, that is a powerful antimicrobial agent. All these factors consider challenging to analyze the samples from the stomach (Nardone & Compare, 2015).

A highly acidic condition of the stomach inhibits the colonization of bacteria. However, this does not mean that there are no bacteria in these harsh environment. For example, facultative or obligatory acidophiles bacteria live at low pH (less than 4) (Nardone & Compare, 2015).

The gastric transit time in eight healthy subjects with the magnetic tracking system ranged from 5 to 133 minutes, with an average of 56 minutes (Worsoe et al., 2011). Transit time is affected by the texture of foods (liquid food needs less time to transit compared to solid and uncooked foods), nutrient composition (longer transit time for fats and carbohydrates), energy density (higher for high-energy diets), and osmolality (higher osmolality for monosaccharides than polysaccharides). The acidic gastric juice inhibits the development of ingested microbes, demonstrates the reduced concentration of less than 10^3 microbial cells per

ml of gastric content. A survey that analyzed 23 samples of gastric mucosal biopsy by 16S rRNA gene sequencing detected a 128 varied phylotypes including phyla Bacteroidetes (35 phylotypes), Fusobacteria (10 phylotypes), Actinobacteria (12 phylotypes), Firmicutes (36 phylotypes), Proteobacteria (32 archaeal phenotypes), and secondary components of the other phyla (Bik et al., 2006).

A high quantity of bacterial strains that are acids-resistant are found in the stomach and include strain of *Neisseria*, *Lactobacillus*, and *Streptococcus*. More than 65% of the phylotypes specified in the stomach are also found in samples taken from the mouth. So, bacterial species such as *Clostridium*, *Veillonella*, and *Lactobacillus* present in gastric juice may be transient (Nardone & Compare, 2015).

Delgado and colleagues (2013) examined the gastric biopsy and gastric juice samples from 12 healthy people by culture and thermal sequencing and established that the dominant genera were *Lactobacillus*, *Streptococcus*, and *Propionibacterium*. Although these studies included communities from different geographical locations (African Americans, Hispanics, Chinese, and Europeans), the stomach microbiota at the taxonomy and genus level together was the same in all populations, albeit to a great degree of inter-subject variation (Delgado et al., 2013).

1.2.2 Small intestine

The small intestine has a shorter food transit time, existence of bile and digestive enzymes makes the surrounding environment inappropriate for microbial growth. The microbiota of the small intestine shows lower taxonomic diversity but higher inter-subject variation than oral or colonic microbiota. *Streptococcus*, *Enterococcus*, *Bifidobacterium*, and *Lactobacillus* dominate the gut microbiota that effectively improves metabolic pathways concentrated around rapid absorption and transformation of simple carbohydrate (CHO). Studies conducted on obesity and gut microbiota center on the differential bacterial efficiency of the small intestine for oxidation of fatty acid and carbohydrate, with the resulting cholecystokinin-mediated reactions on the satiety reflex, while the existence of Lactococcus spp. in this fraction of the intestine is linked with the metabolism of fats and carbohydrates (Mailhe et al., 2018).

A large part of microorganisms in the small intestine are facultatively anaerobic. The levels of bacterial cells increase from the duodenum to the terminal ileum from about 10^4 to 10^8 per ml of intestinal content (Blaut, 2018; Booijink et al. 2010).

Bacteria associated with mucosa in the distal part of the small intestine and colon are phyla Firmicutes and Bacteroidetes but appear in different percentages. Isolating the mucosal-associated bacteria from biopsy samples, resulted in *Helicobacter* (Proteobacteria), *Lactobacillus* (Firmicutes), and *Veillonella* (Firmicutes) in the proximal intestine. While

in the duodenum, jejunum, or ileum, the most abundant bacteria are *Corynebacteriaceae* and *Actinomycinaeae* (both Actinobacteria), *Bacillus* (Firmicutes), and *Streptococcaceae* (Firmicutes), a high percentage of Bacteroidetes, and *Lachnospiraceae* (Firmicutes) are in the colon (Dieterich et al., 2018).

A recent study conducted on 30 liver cirrhosis patients and 28 healthy subjects compares the microbiota in the duodenal. They found that the duodenum contains the genera *Fusobacterium, Brevibacillus, Megasphaera, Prevotella, Porphyromonas, Veillonella, Leptotrichia, Atopobium, Gemella, Neissphaera*, and *Haemophilus* (Chen et al., 2016).

1.2.3 Colon and feces

Due to the relatively long colonic transit time of 35 hours, the microorganisms in the colon have more time to replicate (Chen et al., 2016).

The ion and water absorption through the transit also participates in raising the bacterial density from the cecum (10^8 ml^{-1}) to the distal colon (10^{11} mL^{-1}) (Sender et al., 2016).

The large intestine includes the cecum and colon, contains a high level of saccharolytic anaerobic bacteria such as *Prevotellaceae*, *Bacteroidaceae*, *Ruminococcaceae*, *Rikenellaceae*, and *Lachnospiraceae*. The small intestinal microbiota is responsible for amino acid metabolism and simple

carbohydrates, while the large intestine appropriate for complex sugars fermentation (Li et al., 2020).

While higher species abundance in the human gut, 16S rRNA gene sequencing combined with only a low proportion of the 92 known bacterial phyla with cultured representatives: Verrucomicrobia, Bacteroidetes, Firmicutes, Cyanobacteria, Actinobacteria, and Proteobacteria. These phyla highly differ in their relative contribution to bacterial cells in microorganisms. In a study conducted on 18 people, including monozygotic twins and their mothers, Proteobacteria, Actinobacteria, Firmicutes, and Bacteroidetes members were established to represent about 95% of bacterial cells in the small intestine (Blaut, 2018).

1.3 Microbiota in early life

1.3.1 Establishment of the gut microbiota after birth

Amniotic membranes surround the fetus's body that separates it physically from the uterine cavity and maintains a sterile environment before birth (Haller, 2018).

At birth, the first microbial colonization begins. This process starts when the amniotic sac disrupted, the sterilization is lost, the infant goes down to the birth canal, and he is exposed to the initial phase of bacterial colonization. After birth, there are rapid changes in microbiota composition up to three years of age (Sarkar et al., 2020).

The mother is consider a substantial source of gut bacteria for the infant, in a concept known as perpendicular transportation. Bacteria are transferred to the child perpendicularly from the mother vaginal microbiota, fecal microbiota, and breast milk. In stool samples of infants born vaginally, it was found that more than half of the bacterial species existing in the infant's gut at one day of life derived from the mother, most of them from the mother's gut but also from the skin, vagina, and oral cavity in a cohort study of 25 Italian infants, and the proportion of species shared between mother and infant is up to 72% in a cohort of 98 Swedish infants in the first few days after delivery (Wang et al., 2020).

Studies have specified that the mode of delivery will impact gut microbiota in early life. The infant gut microbiota is very similar to the one exposed during delivery. In infants born vaginally, the intestinal microbiota is similar to their mother's vaginal microbiota, which is *Prevotella*, *Sneathia*, or *Lactobacillus*, while the microbiota of infants born by cesarean section is more similar to the microbiota present on the mother skin, which is dominated by *Propionibacterium*, *Staphylococcus*, and *Corynebacterium*. In addition, studies demonstrated that a delayed colonization by *Bifidobacterium* and *Bacteroides* at one month after birth and an increased the abundance of *Clostridium difficile* in the microbiota of infants born by cesarean section. In addition, the same studies have showed that infants born by elective cesarean section have an especial low bacterial diversity (Tanaka & Nakayama, 2017).

The enteric microbiota composition of infants is highly affected by diet. Many studies showed that Bifidobacterium and lactic acid bacteria (LAB) are more common in the microbiota of breastfed infants. Infants artificially fed have higher diversity in the microbial community, including *Clostridia*, *Bifidobacteria*, *Streptococcus*, *Bacteroides*, and higher abundance of facultative anaerobic bacteria. Facultative anaerobic bacteria include *Enterobacteriaceae*, *Streptococcus*, and *Staphylococcus* (Wall et al., 2009).

The gut microbiota of infants tends to have less diversity than adults, and it is also more dynamic with fast evolution during the first six months of life (Wang et al., 2020).

The newborn gut is an aerobic environment; only aerobe and facultative anaerobic bacteria, such as *Enterobacteriaceae*, can grow. However, within several days, the lumenal intestine becomes anaerobic, allowing colonization of strictly anaerobic bacteria, such as *Clostridium*, *Bacteroides*, and *Bifidobacterium*. The infant microbiota is similar to the mother skin and vaginal microbiota, with predominant *Streptococcus*, *Bifidobacteria*, *Clostridium*, *Enterococcus*, and *Lactobacillus*, during the first few weeks (Arrieta et al., 2014).

1.3.2 Factor influencing the infant microbiota

A wide range of factors can impact the gut microbiota composition, included the way of delivery (vaginal or Caesarean delivery), antibiotic use, eating pattern (formula feeding or breastfeeding), gestational age, and environment factor (Marques et al., 2010).

1.3.2.1 Mode of delivery

The mode of delivery is the main factor that forms the microbiota of infants. In this regard, cesarean delivered infants have an intestinal microbiota that differs from infants born vaginally, both in colonization timing and structure (Wall et al., 2009; Chong et al., 2018; Yang et al., 2016).

After birth, in infants vaginally delivered the first bacterial exposure is from the mother's vagina and GI tract. Cesarean delivered infants do not expose to the vaginal and intestinal bacteria, so they obtain microbiota from the skin of mothers and the hospital environment (equipment, other infants, air, and workers) (Marques et al., 2010).

Cesarean section is considered a surgical intervention conducted to save the life of the baby and the mother in case of poor fetal blood circulation, birth canal blockage, infection, or severe bleeding. The cesarean section requires maternal anesthesia and antibiotics treatments to avoid infection. Studies reported that the early intestinal microbiome of cesarean delivered infants significantly varies through the first months after delivery (Backhed et al. 2015; Dogra et al. 2015; Hesla et al. 2014; Jakobsson et al. 2014; Penders et al. 2013). It displays a low number of *Bacteroides* spp., *Bifidobacterium* spp., a lower variety of the phylum *Bacteroidetes*, and increase in the *Enterobacteriaceae* numbers (Dominguez-Bello et al. 2010).

In the first week of life, the gut microbiota of vaginally delivered infants distinguished by high levels of *Bacteroides* and *Bifidobacterium*. While in

infants born by cesarean section, the most abundant bacteria is *Clostridium* (Hill et al., 2017).

A study analyzed the stool sample of 98 infants (15 delivered by cesarean section) by shotgun sequencing. The results report that the fecal microbiota in cesarean section is enriched in *Haemophilus influenzae*, *Haemophilus aegyptius*, *Haemophilus parainfluenza*, *Haemophilus haemolyticus*, *Staphylococcus saprophyticus*, *Staphylococcus lugdunensis*, *Staphylococcus aureus*, *Enterobacter cancerogenus*, *Enterobacter hormaechei*, *Streptococcus australis*, and *Veillonella parvula*, *Veillonella dispar*. The first colonization for these infants occurs from oral, skin microbes, and bacteria from the external environment during birth. Moreover, the microbiota of infants born vaginally was composed of *Bifidobacterium*, *Escherichia*, *Bacteroides*, and *Parabacteroides* ($p < 0.05$). The variation between delivery patterns constantly decreased at four months and then 12 months, but the cesarean delivered infant microbiota maintain more diverse compared to the vaginally delivered infants (Bäckhed et al., 2015).

1.3.2.2 Infant diet

Feeding methods include breastfeeding and formula feeding, which highly influence the gut microbiota development in early life. Breast milk contains carbohydrates (CHO), proteins, and fats, in addition to endocannabinoids and immunoglobulins. The human milk oligosaccharides (HMOs) include galactooligosaccharide (GOS), which is considered one of the main

components of breast milk. Galactooligosaccharide (GOS) is partially digested in the small intestine and mainly arrives at the colon. Fermentation occurs particularly by *Bifidobacterium* to produce short-chain fatty acids (SCFA) (Tanaka & Nakayama, 2017). Sakurama et al. (2013) mention that *Bifidobacterium* spp. possesses an enzyme called Lacto-N biosidase used to facilitate galactooligosaccharide assimilation (Sakurama et al., 2013). Matsuki et al. (2016) mention that with an increase in the number of *Bifidobacterium* in the infant gut, the number of human milk oligosaccharides (HMOs) in the stool decreased, and the quantity of lactic acid and acetic acid increased in infants at one month of age (Matsuki et al., 2016).

Bacterial transportation from the skin of mothers during lactation is inevitable. Many studies back up the entero-mammary pathway theory, where bacteria from the mother's intestine may arrive at the mammary glands throughout the macrophages and dendritic cells (Arrieta et al., 2014).

Several studies indicate that the microbiota profile for breastfeeding infants is dominated by *Bifidobacterium*, in addition to a small number of other anaerobic and facultative anaerobic bacteria. The colonization of *Bacteroides* and *Bifidobacterium*; is believed to be stimulated by the presence of human milk oligosaccharides (HMOs), the most common carbohydrate component of breast milk. Children lack the enzymes needed to digest human milk oligosaccharides (HMOs), causing them to move into

the colon, where they have thought that acts like prebiotics, increase the growth of *Bacteroides* and *Bifidobacterium*. Moreover, in breastfed infant gut contains more *Lactobacillus* and *Streptococcus* (Yang et al., 2016).

1.3.2.3 Preterm delivery

In premature infants, less than 33 weeks of gestational age, the gut microbiota has lower diversity, due to that most infant delivered by cesarean section, thus they never come into contact with the mother's vaginal microbiota; another cause includes very low weight at birth, so they cannot be fed by breastfeeding, but only by using sterile methods (such as parenteral nutrition). Studies conducted on the gestational age reported that a higher level of facultative anaerobes such as *Enterobacter*, *Lactobacillus*, and *Enterococcus* was found in preterm delivery infants. Full-term infants appear to have strictly low levels of anaerobic bacteria such as *Atopobium*, *Bifidobacteria*, and *Bacteroides*. Other researchers mention that *Firmicutes* and *Proteobacteria* are abundant in the preterm infant gut (Kumbhare et al., 2019).

1.3.2.4 Antibiotic use

Antibiotic therapy is one of the most popular practices in neonatal intensive care units (NICUs) to treat and prevent sepsis and infection (Zwittink et al., 2017).

Antibiotic use in early life has a great impact on the growth of the gut microbiota. The use of antibiotics in infants alters the composition of the

microbiota towards a higher richness of *Proteobacteria* and a lower level of *Actinobacteria*, reduces the general microbial diversity of the infant gut, picking out drug-resistant strains of *Escherichia*, *Enterococcus*, *Klebsiella*, and *Enterobacter*. Not only the receiving of antibiotics, but also the length of the therapy has an impact on the gut microbiota. For example, there is a decrease in the diversity of bacteria by increasing the duration of the therapy period in preterm infants (Henderickx et al., 2019).

In addition, the restoration period from the lower richness of Bifidobacterium was longer for preterm infants who consumed antibiotic therapy for a long time (5 days) compared to those who consumed the therapy for a short time (3 days). The antibiotic impact is visible at least two months after the therapy course (Henderickx et al., 2019).

1.3.2.5 Environment

As the newborns grow up, environmental factors such as family lifestyle and geographical place also affect the microbiota composition. Infants in Western countries are growing in a more hygienic condition which decreases the exposure to disease-causing microbes. In contrast, children who live in developing countries have higher exposure to bacteria, which influence the gut colonization process. Few studies were conducted to compare the gut microbiota of infants from developing and developed countries; moreover, colonization of intestinal bacteria such as *Escherichia coli* is known to be delayed in infants from developed countries compared to those from developing countries. The first one are colonized by a small

number of bacterial strains, with a turnover rate lower than that distinguished in infants from developing countries. Even in the developed world, geographical and ethnic differences have been shown to influence the colonization methods of intestinal bacteria in infants. (Bharadia et al., 2020).

The exposure to the hospital environment comprises the following:1) being with family members and the presence of health workers, 2) geographic location, 3) hospital nursery and neonatal intensive care unit (NICU), 4) and air quality, all of these together may affect the infant microbiota. Although the neonatal intensive care unit (NICU) environment is regularly sterile, commensal and pathogenic bacterial species are present in these places. *Enterococcus*, *Neisseria*, *Streptococcus*, and *Staphylococcus* are usually present on the top of medical tools such as catheters, feeding tubes, pacifiers, and stethoscopes (Sarkar et al., 2020).

A study conducted in Europe reported that infants from Northern regions have several *Clostridium* spp. and *Atopobium* spp. and a higher number of Bifidobacterium spp. However, Southern infants have a higher richness of *Bacteroides*, *Eubacterium*, and *Lactobacillus*. A study reported that the microbiota composition significantly different between German and Finnish infants: higher percentage of *Bifidobacterium* spp. is present in Finnish infants, while German ones show higher richness in *Akkermansia muciniphila*, *Bacteroides, and Prevotella*. Another study, in Malawian infants, showed a higher number of *Clostridium histolyticum*,

Bifidobacterium spp., *Bacteroides, and Prevotella* compared with Finnish infants at six months of age (Rodríguez et al., 2015).

1.3.3 Dysbiosis and disease

Dysbiosis is known as an unbalance in the gut microbiota composition, that can appear as a decrease in diversity of microbes or loss of butyrate-producing bacteria such as Firmicutes, beneficial bacteria such as strains of *Bacteroides*, and an increase in opportunistic pathogens (symbiotic bacteria that become pathogenic under specific situation), including Proteobacteria, such as *E. coli* (Humphreys, 2020).

Dysbiosis categorized into several types includes 1) loss of diversity of the gut microbiota, 2) loss of beneficial microorganisms, and 3) increase in the abundance of potentially harmful microorganisms. It has been found that these types can happen at the same time and are not mutually exclusive (DeGruttola et al., 2016).

Changes in the composition and diversity of the gut microbiota were reported in different diseases, and a comparative analysis detected a significant difference between healthy and patients with immune diseases, cancer, inflammatory bowel disease (IBD), metabolic diseases. Notably, multiple elements can demonstrate these differences in the microbiota, but a direct causal contribution was never confirmed (Hornef, 2018; DeGruttola et al., 2016).

One case in which the microbiota is thought to participate in the etiology of the disease is neonatal necrotizing enterocolitis (NEC) (Berman & Moss, 2011). This case is established nearly in preterm infants (infants born less than 37 weeks of gestation) and distinguished by the presence of necrosis and acute inflammation in the colon tissue. Regardless of the surgical intervention, it is correlated with a greater number of morbidity and mortality. Interestingly, its prevalence and acuteness raise with lowering the gestational age of the premature infants and generally start with a delay of 1-2 weeks after birth, that is, after the colonization of intestinal bacteria. It is believed that unsuitable immune stimulation of the immature intestinal mucus membrane by the microbiota leads to tissue inflammation. In every case, it is thought that several immune-regulating mechanisms are able to protect the mature infant mucosal membrane from unsuitable microbiota-promote immune stimulation are unavailable or impaired in the preterm gut (Hackam et al., 2013). Also, Roze and colleagues reported a significant change in microbiota in preterm children, and some of these changes correlated with necrotizing enterocolitis (NEC) (Roze et al., 2017). For example, there is a greater number of *Enterobacteriaceae* demonstrated in the preterm human intestine, and the component of external cell membrane of Gram-negative bacteria, lipopolysaccharide (LPS), and its TLR-like receptor (TLR) have been specified for propulsion mucositis in animal models of necrotizing enterocolitis (NEC) (Egan et al., 2015).

In Crohn's disease (CD), dominant dysbiosis is characterized as being correlated with five species of bacteria, among which modulation in the

availability of Faecalibacterium *prausnitzii* is connected with the extension of disease alleviation. Feeding with this microorganisms, there is a therapeutic effect for colitis in experimental models (Carding et al., 2015).

The gut microbiota has a significant effect on the liver; because microbial output permanently passes to the liver via the portal vein. Usually, a small number of the intestinal microbes and their metabolites that enter the liver are removed by Kupffer cells. When intestinal epithelial cell tight junction function is disrupted, bacterial transportation and the entry of bacterial metabolites into the liver may cause liver disease. A recent study conducted on animal models observed that gut microbiota participates to age-dependency for hepatitis B virus elimination. Sterilization of intestinal microbiota at age 6 to 12 weeks using antibiotics prohibited the adult mice from relieving the hepatitis B virus. In people with cirrhosis, the composition of the gut microbiota varies with higher abundance of *Streptococcaceae* and *Enterobacteriaceae*, while that of other bacteria such as *Lachnospiraceae* and *Bifidobacteria* is lower. Impaired bile excretion and hypertension can lead to dysbiosis and affect the function of the normal liver. Hepatic encephalopathy is a common complication in patients with severe cirrhosis. Hepatic encephalopathy is not caused by the damaging of organs but by toxic substances released by gut bacteria. *Porphyromonadaceae*, *Enterobacteriaceae*, and *Alcaligenaceae* are closely correlated with inflammation and cognition in hepatic encephalopathy patients. It is also reported that the gut microbiota changes the risk of hepatocellular carcinoma (HCC) in animal experiments. Gut colonized by

Helicobacter hepaticus enhances aflatoxin- and hepatitis B virus that induce hepatocellular carcinoma (HCC). *Enterobacteriaceae* did not cause hepatocellular carcinoma via bacterial transportation to the liver nor induce hepatitis. Alternatively, gut Helicobacter hepaticus stimulates the nuclear factor-κB regulating networks with innate immunity and Th1 in both liver and the lower GI tract (Lu & Ni, 2015).

The lower colonization resistance may also participate in the markedly various spectra of pathogenic organisms affecting infants. The most popular causative factors of infant sepsis, *Escherichia coli*, *Listeria monocytogenes*, and *Streptococcus*, are rarely seen in adults. The main reason for gastroenteritis in infants are rotavirus, enteropathogenic and intestinal hemorrhagic *Escherichia coli*. These pathogenic bacteria rarely induced diseases in adults (Kotloff et al., 2013).

Allergic and autoimmune disease incidence in developed countries has raised constantly over the past 30 years. The lifestyle in the modern Western population strongly influences the composition of the gut microbiota. A recent study noted a higher abundance of *Bacteroides* species in Finnish and Estonian children microbiota compared Russian children. (Vatanen et al., 2016).

1.4 Mediterranean diet

Researchers defined the Mediterranean Diet as a dietary pattern characterized by a high consumption of vegetables, fruits, legumes, whole grains, and their products, extra-virgin olive oil, and nuts. Consumption of

low to moderate amount of dairy products, fish, and a limited quantity of red and processed meat, in addition to red wine. This diet is low in saturated fats, while rich in unsaturated fats (such as olive oil), antioxidants (particularly vitamin C, vitamin E, and polyphenols), fiber, and glutathione (Amati et al., 2019; Chatzi et al., 2017).

1.5 Diet and microbiota

Diet is considered a major factor influencing the health of the gut microbiota. Growing evidence indicated that diets high in plant foods rather than animal foods appear to be a healthy option for disease prevention (Chiavaroli et al., 2019; Kelly et al., 2016).

A balanced and diverse diet is necessary to enhance the reservation of diversity and the adequate functioning of a healthy microbiome in the gut (Garcia-Mantrana et al., 2018).

The gut microbiome is substantially affected by the components, amount, and timing of its host's diet. Increasing proof indicates that feeding time has a dominant impact on immune functions and metabolic in microbiome-dependent and independent behaviors. In a specified person, significant variability is observed when eating identical meals at distinct times of the day (Leshem et al., 2020).

Actually, short-term changes in dietary habits from a high-fat/low-fiber diet to a low-fat/high-fiber diet cause reproducible alterations in the gut microbiota that are not permanent. In contrast, the usual diet in the long

term is the essential factor that shape our gut microbiota (David et al., 2013).

Comparison of gut microbiota in Western and agricultural populations may aid in understanding how dietary changes affect gut microbes. All of these studies have continuously established that the composition of the gut microbiome vary significantly between Western and urban populations who consume a diet rich in fat and protein, and rural residents still eating an agrarian diet, with low consumption of animal products and high intake of vegetables, fruits, roots, and fibrous tubers. Most of these studies show a lower microbial diversity in Western societies (Clemente et al., 2015).

People who consume Western diets have a large tendency to lose microbial diversity in the gut. The gut microbiota composition also underwent particular alterations, characterized by a rise in the richness of the *Enterobacteriaceae* family, Firmicutes phylum, and a decrease in the *Prevotella* genus and Actinobacteria phylum (Moles & Otaegui, 2020).

The high-fructose diet changed the gut microbiota and degraded the intestinal barrier in in-vivo experiments. The intestinal metabolite profile correlates with fructose feeding results in lower bacterial diversity and appears to have a greater possibility in terms of encouraging host metabolic distraction. The results also demonstrated that high-fructose diets cause inflammation and metabolic disturbances due to alteration in the content of gut microbiota and increase the permeability of the intestine. Mice consuming a diet containing 60% of fructose, show changes in the

intestinal microbiota and integrity of the intestinal mucosa. However, there is only a little experimentation on the effect of low-dose fructose on the gut microbial population and the subsequent impact on the function of the intestinal barrier and inflammatory response. High fructose consumption caused fat accumulation in the liver, infiltration of inflammatory cells in the pancreas and colon, and higher richness of *Parasutterella*, *Blautia*, *Marvinbryantia*, and *Lachnospira* in colon microbiota (Wang et al., 2020).

A study conducted on 12 men comparing a vegan diet with a lacto-ovo-vegetarian diet and a Western diets. They established that the enterococci and lactobacilli in feces were lower in the vegetarian diet, and there is a lower concentration of fecal bile acids in both lacto-ovo-vegetarian and vegetarian (Chen et al., 2018).

The diet function in the adjustment of gut microbiota is demonstrated. De Filippis and colleagues showed that the Mediterranean diet, distinguished by high plant foods components, plays an effective role in shaping the gut microbiota composition. In particular, people who consume a higher proportion of plant foods display a higher abundance of fiber-degrading bacteria and short-chain fatty acids (SCFAs) in their stool. People with low commitment to the Mediterranean diet had a higher level of trimethylamine oxide (TMAO) in their urine.

The high level of commitment to the Mediterranean diet is recognized by increased *Bifidobacteria* and a high proportional level of short-chain fatty acids (SCFA). So, the Mediterranean diet plays a favorable effect on the

gut microbiota, particularly on alpha diversity and microbial metabolic activities (Merra et al., 2020).

1.5.1 Microbial production of beneficial or detrimental metabolites from dietary precursors

Various dietary habits and gut microbiomes enhance the production of distinct metabolites in feces within traditional and Western population. Undigested food components pass to the colon, where they are fermented by the microbial community to produce several metabolites, reflecting the chemically diverse substrates and the various metabolic function of the gut microbiota. All studies have determined the concentration of short-chain fatty acids (SCFAs) in feces. The most common short-chain fatty acid (SCFAs) includes butyric, acetic, and propionic acids, that are produced by bacterial fermentation of indigestible fiber, and are usually present in a molar ratio of about 3:1:1 (Louis et al., 2014).

Short-chain fatty acids(SCFAs) are considered an essential source of energy for human colon cells and correlated with many health-enhancement effects, such as anti-carcinogenic and anti-inflammatory (O'Keefe, 2016).

The Mediterranean diet contains oligosaccharides, some cellulose, gum, and beta-glucans. Fermented fibers are a good source of substrate for bacterial metabolism, yielding to short-chain fatty acids (SCFAs), especially propionate, butyrate, and acetate. Studies recently reported that these metabolites play an essential role in the regulation of lipid

metabolism, immunity, blood pressure, glucose, representing the correlation between microorganisms and human homeostasis (Merra et al., 2020).

Urolithins represent gut microbial metabolites of ellagitannins. Urolithin-A is considered an anti-inflammatory, anti-aging, and antioxidant activity (Roager & Dragsted, 2019).

Glycolytic fermentation occurs in the proximal colon, where most bacteria use carbohydrates rather than proteins. Conversely, in the distal colon the proteolytic fermentation occurs, yielding branched-chain fatty acids (BCFAs) and potentially harmful metabolites such as indoles, phenols (from carboxylation of the amino acid), and ammonia (from amino acid deaminase and urea hydrolysis) (Vernocchi et al., 2020).

A high concentration of branched-chain amino acids in the blood has been correlated with the resistance to insulin and the occurrence of type 2 diabetes (Pedersen et al., 2016).

Phenylacetate, a microbial product of phenylalanine, has been demonstrated to induce steatosis (infiltration of hepatocytes with fat) in rats and primary cultures of the human liver cells (Hoyles et al., 2018).

In the colon, microbial fermentation produces phytochemicals compounds, and some bacteria can alter them to different bioactive molecules. For example, daidzein and soy isoflavone can be converted into equol by several gut bacteria, such as *Eubacterium*, *Bifidobacterium*, *Clostridium*,

and *Faecalibacterium*. Based on several scientific evidence, equol may have anti-cancer properties (De Filippis & Ercolini, 2018).

Sulfide is produced from sulfate-reducing bacteria (such as *Desulfovibrio* spp.) throughout sulfur amino acids and taurine catabolism. Sulfides consider toxic and pro-inflammatory to the colon cells (O'Keefe 2016).

Catabolism by the gut microbiota of choline, phosphatidylcholine, and L-carnitine yields triethylamine (TMA), which is further oxidized in the liver resulting in triethylamine (TMA). It is considered a risk factor in the development of cardiovascular diseases (CVDs) and atherosclerosis (De Filippis & Ercolini, 2018).

Chapter Two
Aims

The main aim of this study is to analyze the adherence to the Mediterranean diet with gut microbiota during pregnancy.

Specific objectives:

- Analysis of the adherence to the Mediterranean diet during pregnancy.
- Analysis of maternal microbiota during pregnancy.
- The association between maternal gut microbiota and the MedD index variation.

Chapter Three

Methods

3.1. Study design and population

The trial was carried out at the University of Naples Federico II and was approved by the related Ethics Committee of the University of Naples Federico II. Each participant provided written informed consent and received no financial compensation. The trial was registered at ClinicalTrials.gov (number NCT0337802). The protocol ended when the last group of participants completed the protocol (Study Start Date: November 2017; Completion Date: February 2020).

We investigated the gut microbiota by fecal DNA in 76 pregnant women (recruited from the University hospital) in response to a Mediterranean diet (n=35) or a control diet (n=41). The commitment to the Mediterranean diet was evaluated by using the 11-unit dietary score and reported as the Italian Mediterranean Index (MD Index) (Agnoli et al., 2011). Fecal samples were self-collected by the participants in sterile containers at different periods during the pregnancy (1^{st}, 2^{nd} and 3^{rd} trimester) and stored at −80 °C until DNA extraction.

3.2. Eligibility Criteria

Participants included in the study were Caucasian adult women, age between 20-35years old, and healthy pregnant women without any allergic disease.

Exclusion criteria included:

- Proven presence of infections during pregnancy and at delivery,
- Twin pregnancy,
- Ongoing malignancies,
- Major gastrointestinal tract malformations,
- Immune-deficiencies,
- Diabetes and other chronic diseases at each organ or apparatus level,
- Chronic intestinal inflammatory diseases,
- Gastrointestinal function disorders,
- Celiac disease;
- History of abdominal surgery with intestinal resection,
- Neuropsychiatric disorders,
- Central nervous system disorders,
- Vegan diet.

3.3. Mediterranean diet index

The Italian Mediterranean diet index score is based on 11 food items: high intakes of 6 typical Mediterranean foods (pasta; typical Mediterranean vegetables such as raw tomatoes, leafy vegetables, onion, and garlic, salad,

and fruiting vegetables; fruit; legumes; olive oil; and fish); low intakes of 4 non-Mediterranean foods (soft drinks, butter, red meat, and potatoes); and moderate alcohol consumption.

If consumption of the typical Mediterranean products was in the 3rd percentile of the distribution, the person received 1 point; all other intakes received 0 points. If consumption of non-Mediterranean foods was in the first percentile of the distribution, the person received 1 point. Alcohol received 1 point for intake up to 12 g/d; abstainers and persons who consumed less than 12 g/d received a 0. Possible scores ranged from 0 to 11. Components and standard portions for optimal scoring of each index are summarized in Table 1.

Table 1: Components, with optimal quantities of components consumed for scoring, of the Italian Mediterranean Index

Food category	Mediterranean diet index
Total grain	Not specified
Pasta	≥3rd tertile (73 g/d)
Whole grain	Not specified
Total vegetable	Not specified
Dark green and orange vegetables and legumes	Not specified
Mediterranean vegetables	≥3rd tertile (162 g/d)
Potatoes	≤ 1st tertile (17 g/d)
Total fruit	Excluding fruit juices, ≥3rd tertile (392 g/d)
Whole fruit	Not specified
Milk product	Not specified
Skimmed milk products	Not specified
Meats, fish, legumes and nuts	Not specified
Nuts and legumes	Legumes ≥3rd tertile (23 g/d)
Fish	≥3rd tertile (38 g/d)
Red and processed meat	≤ 1st tertile (70 g/d)
Oils	Olive oil ≥3rd tertile (30 g/d)
Butter	≤ 1st tertile (1.4 g/wk)
Fatty acids	Not specified
Sodium	Not specified
Energy from solid fat, alcohol and added sugar	Not specified
Soft drinks	≤ 1st tertile (0 g/d)
Alcohol	0.01–12 g/d

3.4. Dietary intervention

Participants randomized in the Mediterranean diet group were provided with a dietary regimen, to increase their adherence level to the Mediterranean diet, maintain balanced daily energy and macronutrients intake, while participants in the control group were instructed to not change their habitual diet. Visits and sample collection were performed at baseline (1^{st} trimester of pregnancy), at 6 (2^{nd} trimester of pregnancy) and 9 (3^{rd} trimester of pregnancy) months.

3.5. DNA extraction

DNA extraction from fecal samples was carried out following the SOP 07 developed by the International Human Microbiome Standard Consortium (www.microbiome-standards.org).

1. Fecal sample (2g) homogenized in 3ml STE (100 mM NaCl, 10 mM Tris-Cl pH 8.0, 1 mM EDTA) or PBS buffer, vortex, and centrifuged for 1 min at 1000rpm

2. Prepare the beads (2ml), collect 600µl of the fecal sample, 250µl guanidinium thiocyanate (protein denaturant), and 500µl of 5% N-Lauroylsarcosine sodium salt (ionic surfactant).

3. Vortex the sample, and incubate it for 60 min at 70 °C (700 rpm).

4. Vortex the sample for 10 min at the max speed.

5. Centrifuge the samples for 1 min at 14000RCF.

6. Collect the supernatant into 2 ml tube.

7. Add polyvinylpyrrolidone powder, mix it well, and vortex.

8. Centrifuge the samples for 3 min at 15000 x g and 4°C.

9. Collect 500µl from each sample put it in a 2ml tube, add 8µl from RNase, vortex, and incubate for 20min at 37 °C (700rpm).

Total DNA was purified by using the NucleoSpin gDNA Clean-up Kit (Macherey-Nagel), according to the manufacture instruction.

10. Add 1500µl of binding buffer to the samples and vortex for 5 seconds.

11. Transfer each sample to one column; this is performed in three steps with each 650µl, centrifuge for 30 sec (14000 x g).

12. Add 700µl of washing buffer and centrifuge for 3sec (14000 x g). Repeat this step 3 times.

13. Dry the membrane by centrifuge samples for 2 min at 14000g.

14. Add 50µl buffer DE (in the center) to elute DNA, wait 2 min, and centrifuge for 1 min. repeat the elution step and pool to obtain a final volume of 100µl.

3.6. Metagenome sequencing and data analysis

Total DNA was sequenced on Illumina NovaSeq platform, leading to 2x150bp, paired-end reads. Human reads were removed using the Human Sequence Removal pipeline developed within the Human Microbiome

Project by using the Best Match Tagger (BMtagger; https://hmpdacc.org/hmp/doc/HumanSequenceRemoval_SOP.pdf).Then, non-human reads were quality-filtered using PRINSEQ 0.20.4: reads with bases having a quality score < 15 were trimmed and those shorter than 75 bp were discarded. High-quality reads were imported in MetaPhlAn 3.0 to obtain species-level, quantitative taxonomic profiles. Use spss to analyze the data.

3.7. Statistical analysis

Correlation analyses, sample clustering, and statistical analyses were carried out in an R environment. Pairwise Wilcoxon tests were used to test the significance of specific bacterial taxa and MD adherence score. Pairwise Spearman correlations were calculated between microbial genera and the MedD index variation. The correlation plots were visualized and clustered in R using the ggplot2 package.

Chapter Four

Results and Discussion

4.1. Results

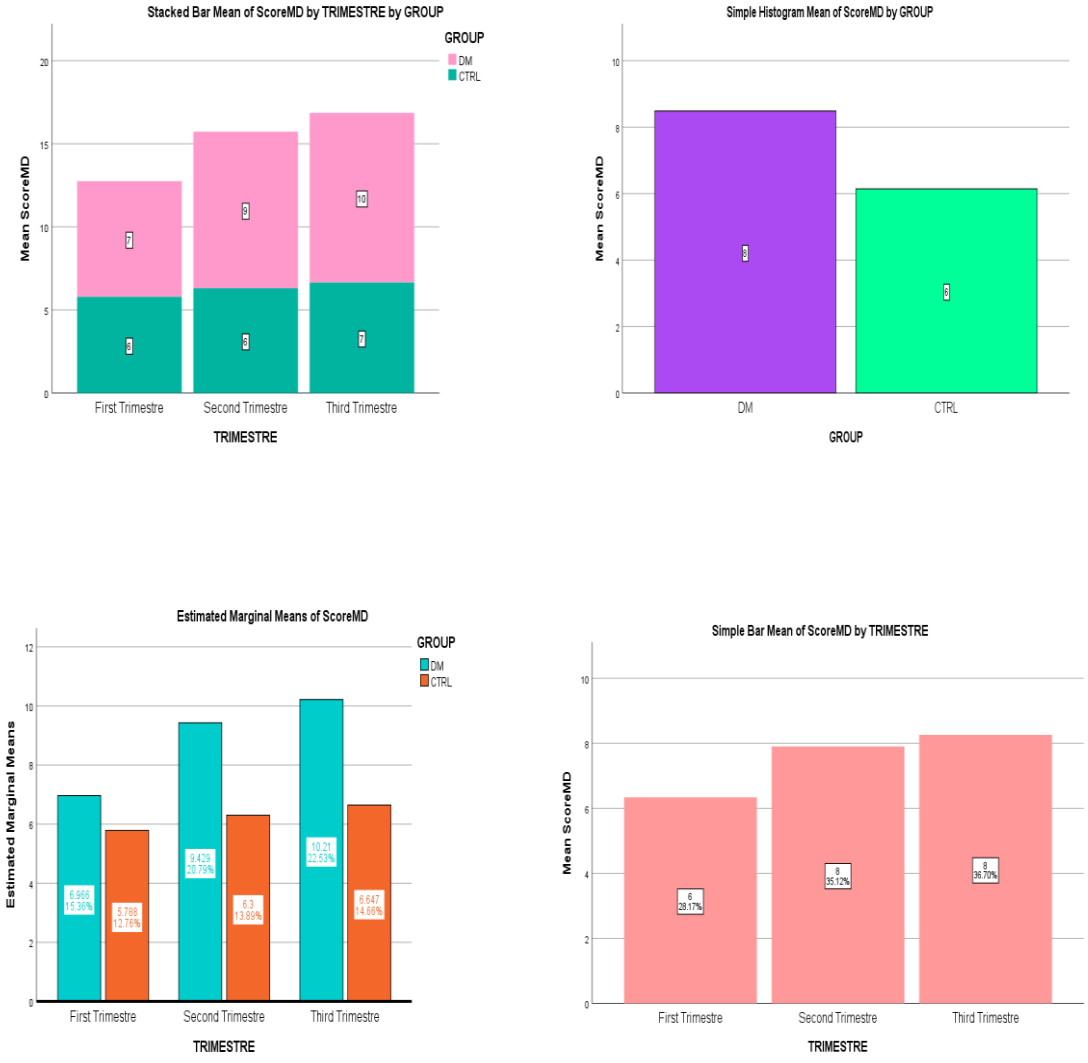

Figure 1: The mean value of Med-score in each group (Med and control group) and in each trimester: at 3 (1st trimester of pregnancy), at 6 (2nd trimester of pregnancy) and 9 (3rd trimester of pregnancy) months.

Table 2: Descriptive Statistics of the study population.

Dependent Variable: MD score				
Trimester	GROUP	Mean	Std. Deviation	N
First Trimester	DM	6.97	1.936	29
	CTRL	5.79	2.012	33
	Total	6.34	2.048	62
Second Trimester	DM	9.43	1.777	21
	CTRL	6.30	1.658	20
	Total	7.90	2.322	41
Third Trimester	DM	10.21	1.369	14
	CTRL	6.65	1.730	17
	Total	8.26	2.380	31
Total	DM	8.48	2.254	64
	CTRL	6.14	1.859	70
	Total	7.26	2.362	134

The mean Mediterranean score was higher in Med-group (mean value=8.26) than the control group (mean value=6.14) and increasing during the pregnancy. However, the increase was significant only in the Mediterranean group, reaching a mean value of 10.21 in the third trimester.

The adherence to the Mediterranean diet increased during pregnancy. The score increased around 7.01% during the first (adherence %=28.17) and second trimesters (adherence %=35.12). While in the third trimester, remain constant (no changes).

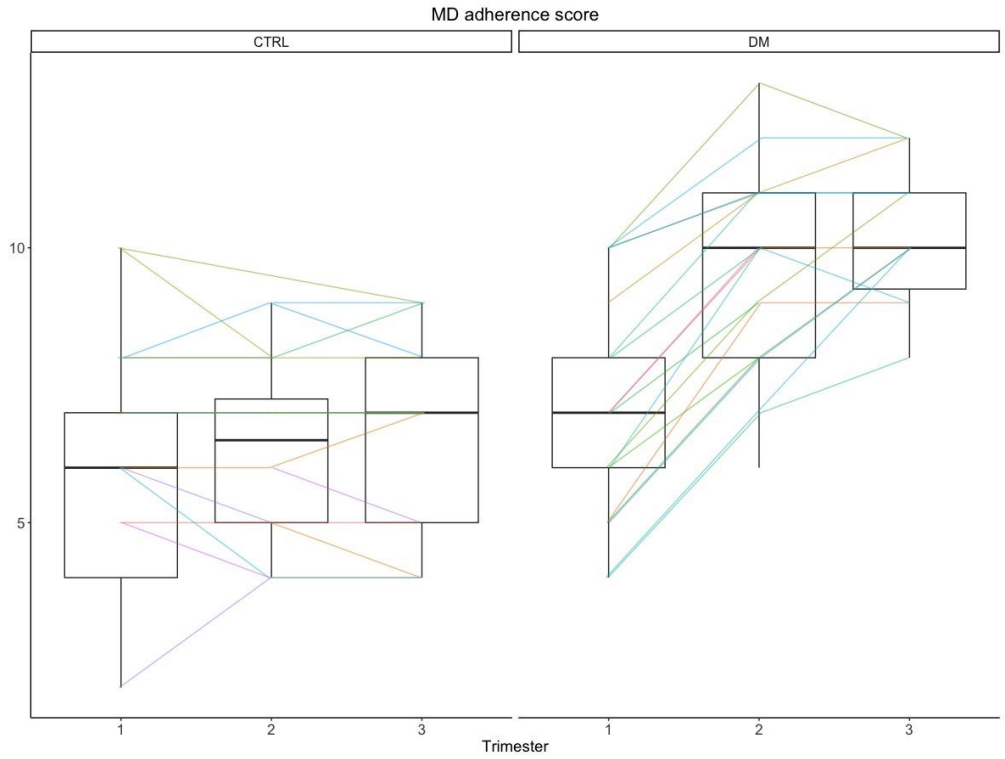

Figure 2: MD index score for controls (Control Diet) or treated subjects (Med-Diet) during pregnancy.

Indeed, we observed higher compliance to the Mediterranean diet in the Med-group during the second trimester of pregnancy compared to the control group.

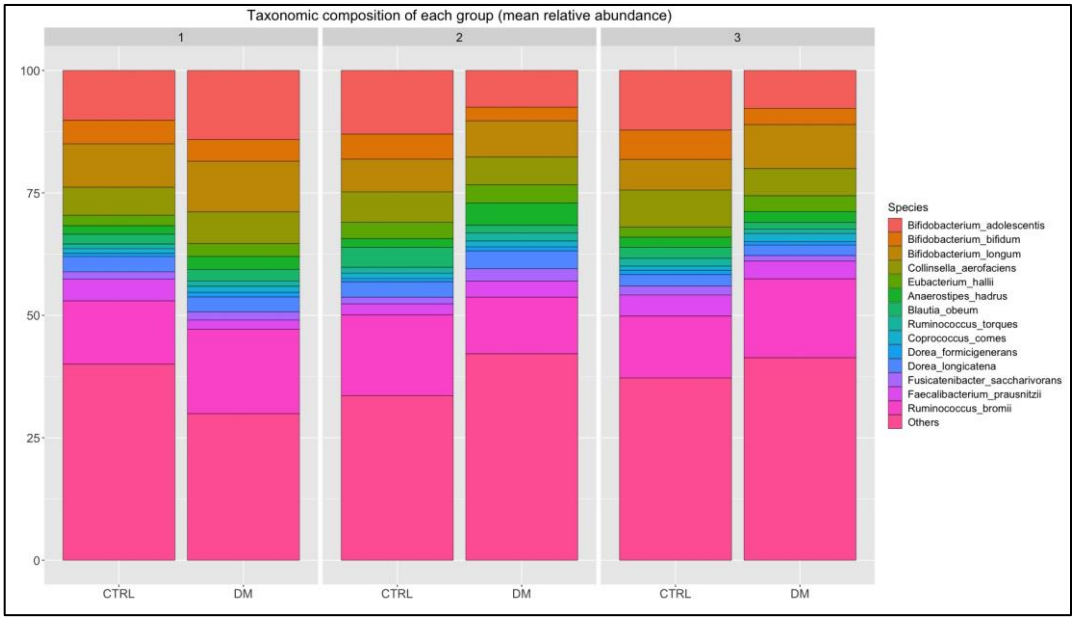

Figure 3: Taxonomic composition of each group (Med and control group) during pregnancy at 1st trimester, 2nd trimester, and 3rd trimester.

We compared the gut microbiome composition in control and Med-diet subjects during pregnancy. Considering the composition at species level, the gut microbiota was mainly composed of *Ruminococcus bromii*, *Bifidobacterium bifidum*, *Bifidobacterium longum*, *Collinsella aerofaciens*, *Bifidobacterium adolescentis* and *Faecalibacterium prausnitzii* in both groups. Higher abundance of *Bifidobacterium longum* and *Ruminococcus bromii* was observed in Med-group compared to the control during the third trimester of pregnancy. The abundance of *Bifidobacterium adolescentis* decreased in Med-group, while increased in control group during pregnancy. The abundance of *Eubacterium hallii* increased in the second trimester, and was higher in Mediterranean group compared to control group.

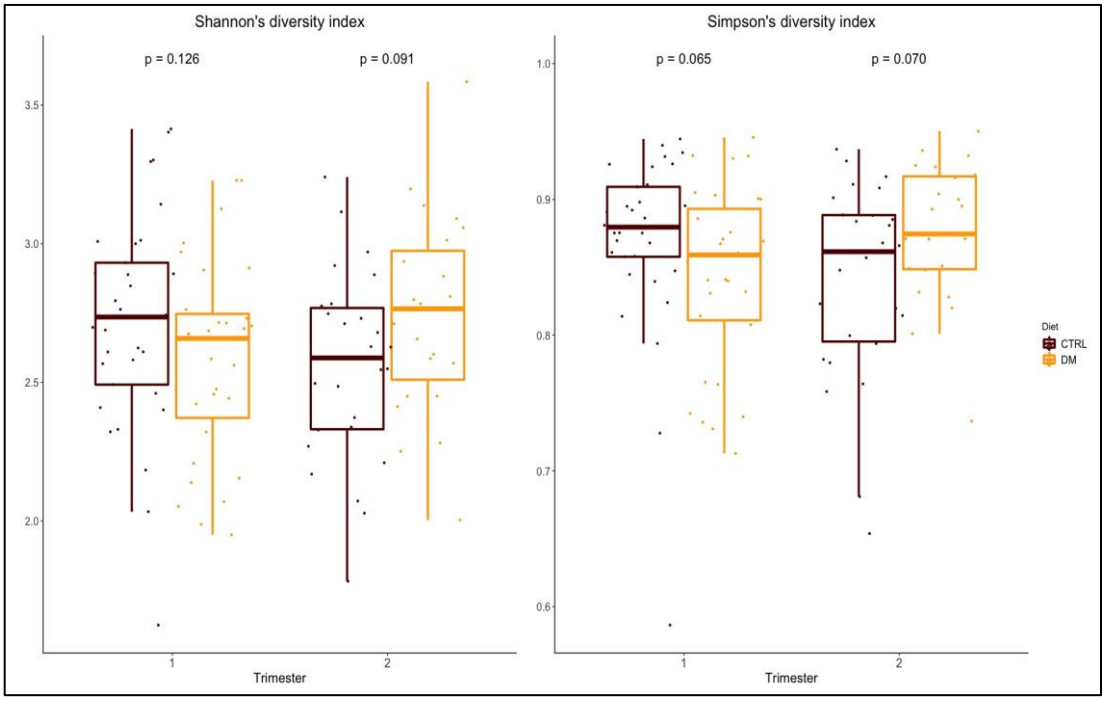

Figure 4: Gut microbiota diversity according the diet during first and second trimester.

Although the Med-group had a lower microbial diversity at the 1st trimester compared with the controls, it increased during the second trimester, as observed by both Shannon and Simpson diversity indices.

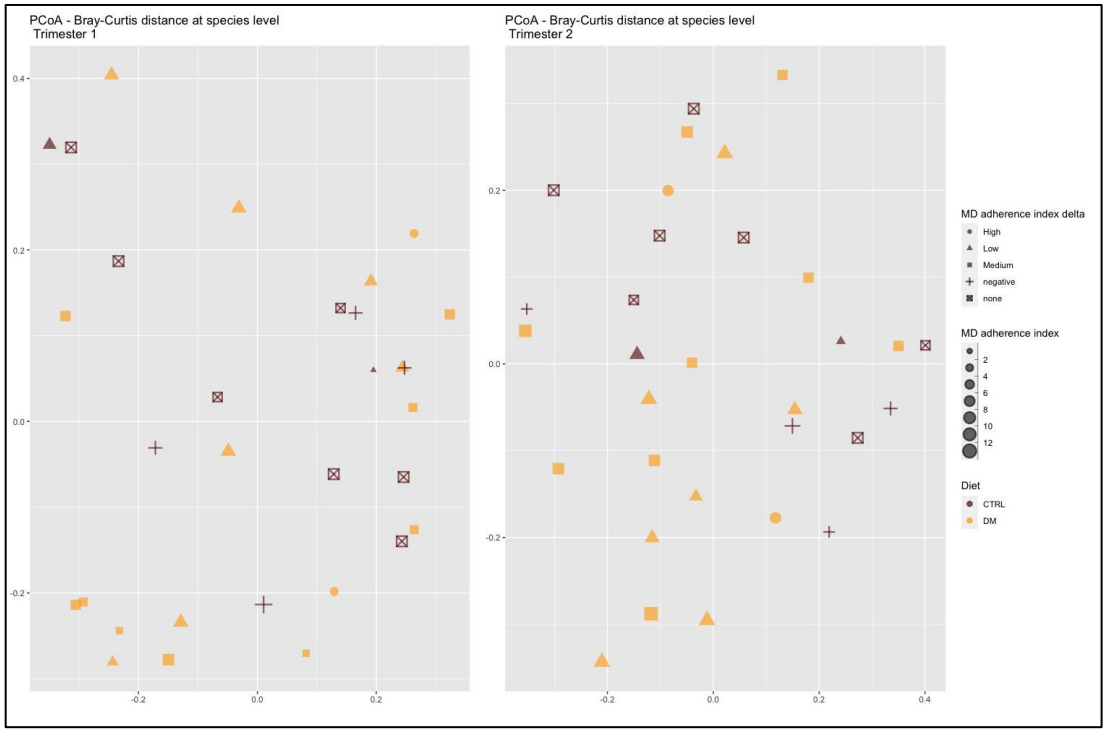

Figure 5: PCoA (Principal Coordinates Analysis) based on the Bray-Curtis distance matrix obtained by the species level microbiota composition. Subjects are separated according to the trimester of pregnancy.

Slight separation of the Mediterranean group compared to the control during the second trimester was observed in the PCoA.

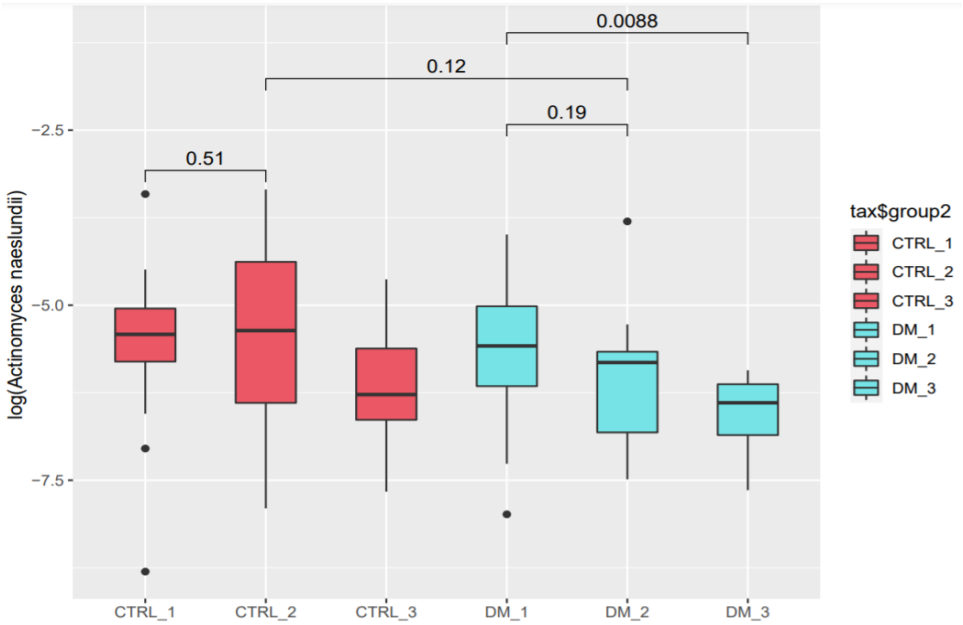

Figure 6: Box plot showing the abundance of *Actinomyces naeslundii* for Mediterranean diet and control group.

In MedD group, a significant decrease (P-value=0.0088) in *Actinomyces naeslundii* abundance was observed at the third semester.

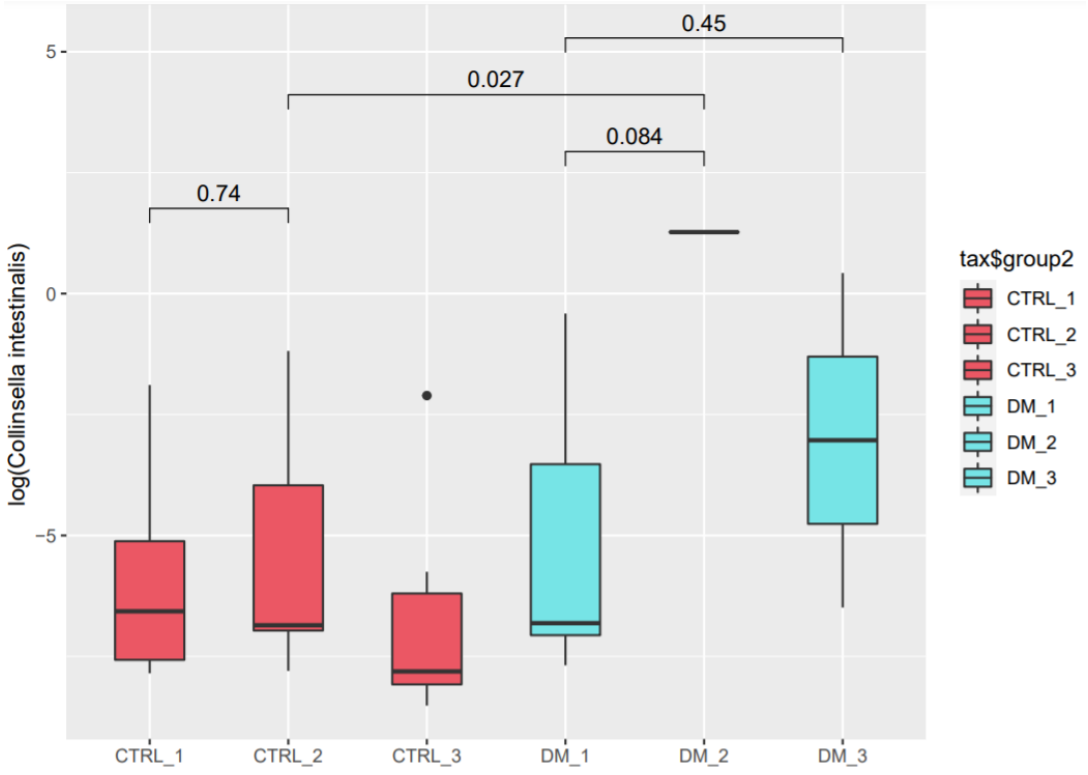

Figure 7: Box plot showing the abundance of *Collinsella intestinalis* for Mediterranean diet and control group.

There is an almost significant increase (P value=0.084) in *Collinsella intestinalis* in MedD-group, while there are no significant changes in the control group (P-value=0.74) during pregnancy.

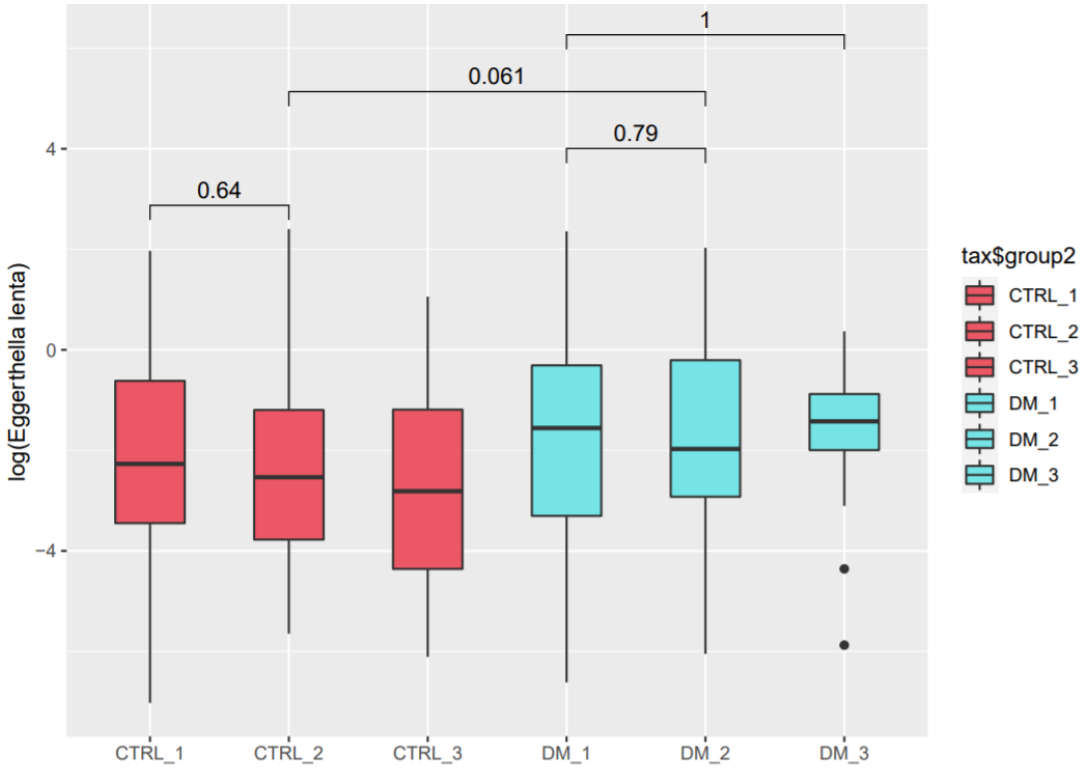

Figure 8: Box plot showing the abundance of *Eggerthella lenta* for Mediterranean diet and control group.

There are no significant changes (P value>0.05) in *Eggerthella lenta* in both groups during pregnancy.

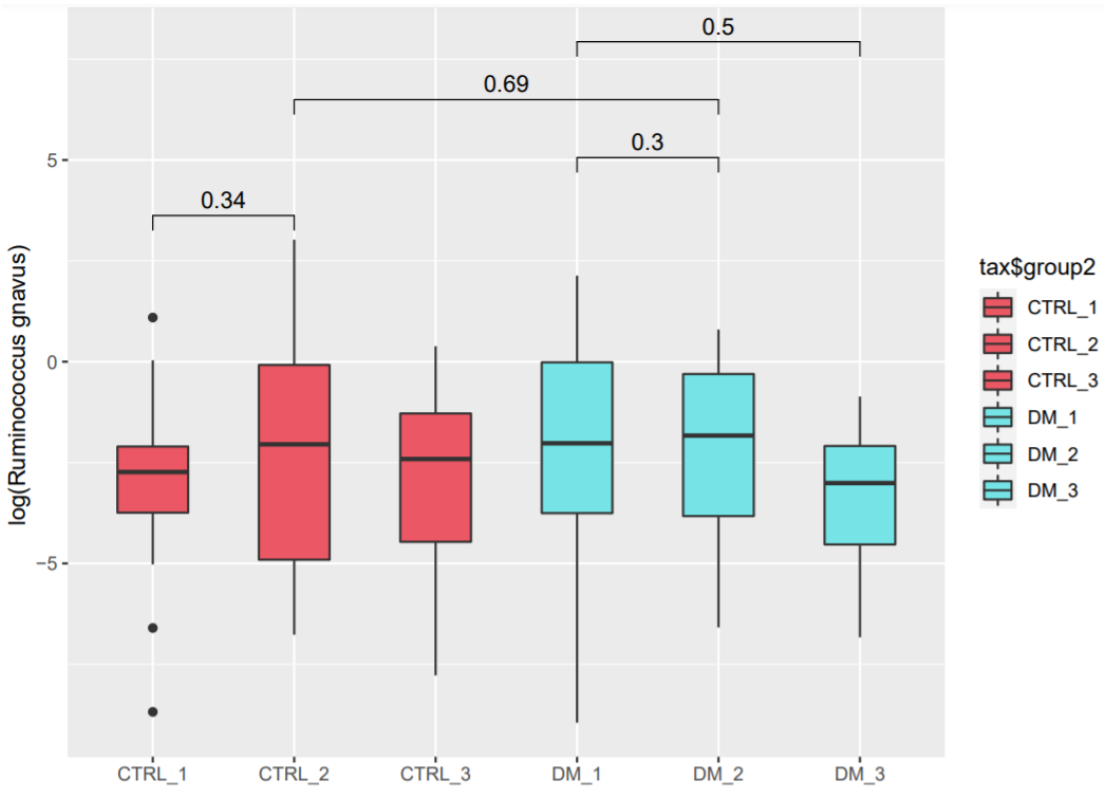

Figure 9: Box plot showing the abundance of *Ruminococcus gnavus* for Mediterranean diet and control group.

There are no significant changes (P-value>0.05) in *Ruminococcus gnavus* in both groups during pregnancy. However, there is a trend in the decrease in *Ruminococcus gnavus* in the MedD group during pregnancy.

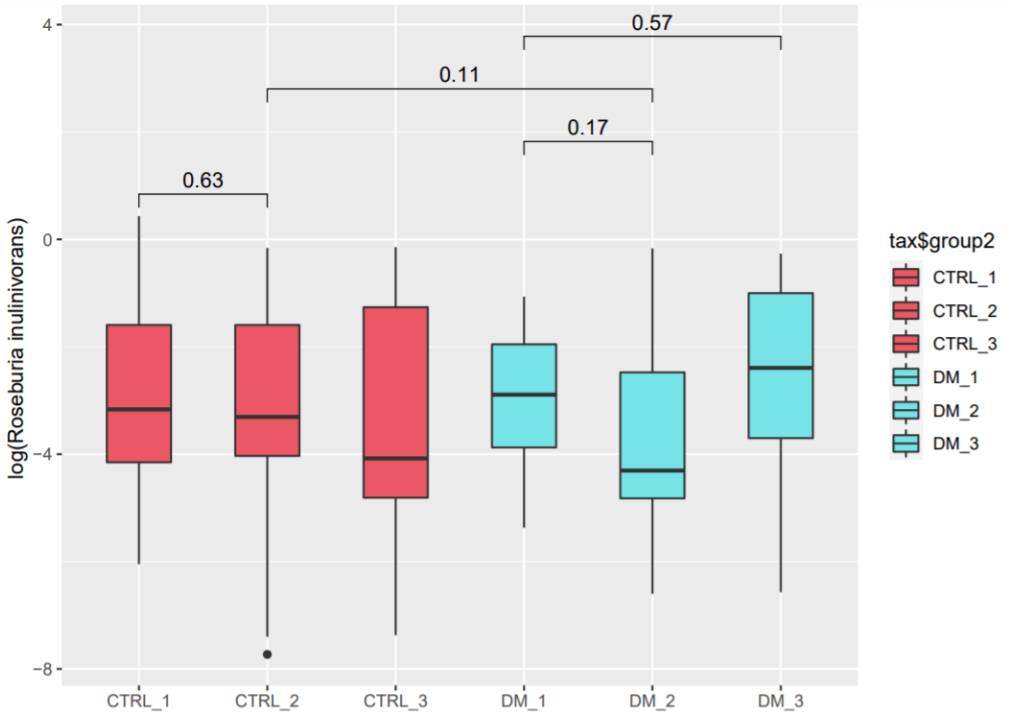

Figure 10: Box plot showing the abundance of *Roseburia inulinivorans* for Mediterranean diet and control group.

There are no significant changes (P-value>0.05) in *Roseburia inulinivorans* in both groups during pregnancy. However, there is a trend in the increase in *Roseburia inulinivorans* in the MedD group during the third trimester.

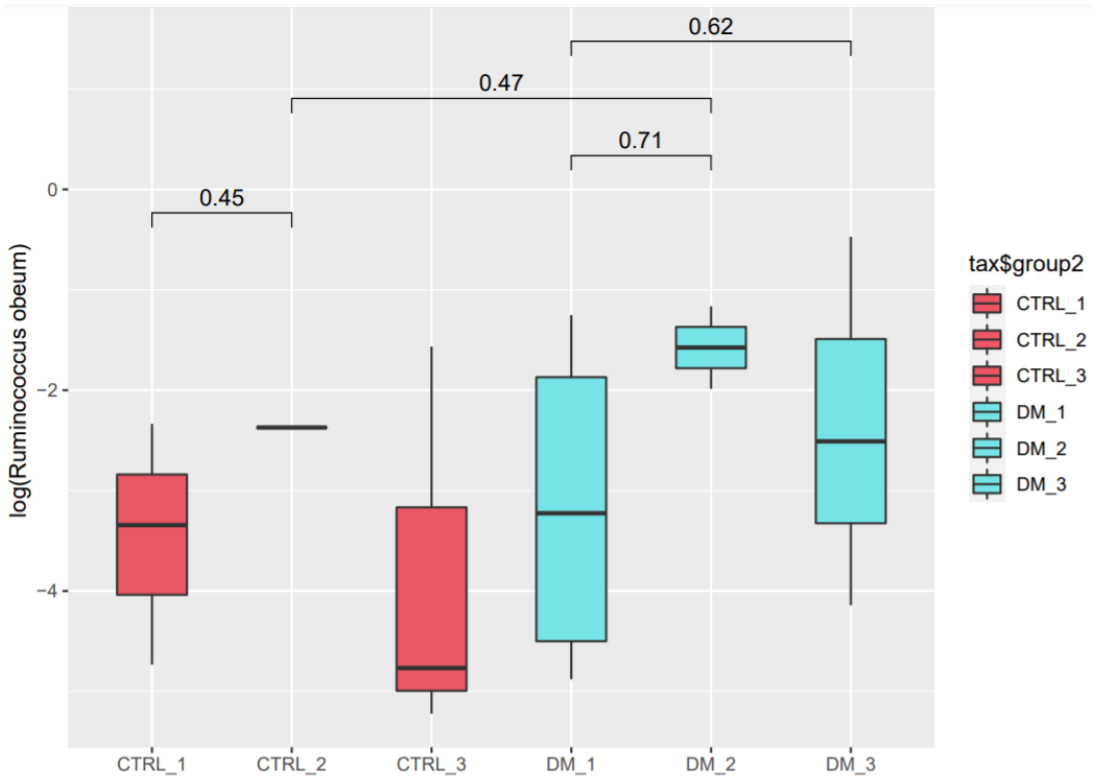

Figure 11: Box plot showing the abundance of *Ruminococcus obeum* for Mediterranean diet and control group.

There are no significant changes (P-value>0.05) in *Ruminococcus obeum* in both groups during pregnancy. However, there is a trend in the increase in *Ruminococcus obeum* in the MedD group during the second trimester, and remain constant in the third trimester.

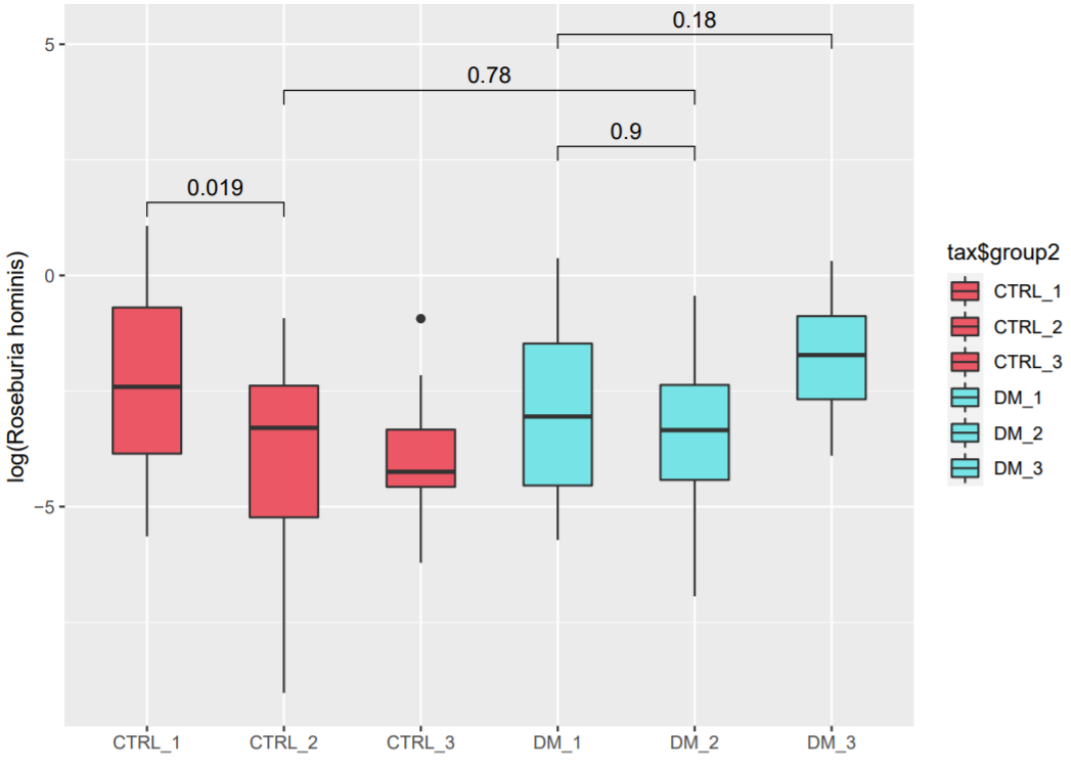

Figure 12: Box plot showing the abundance of *Roseburia hominis* for Mediterranean diet and control group.

There is a significant decrease (P-value=0.019) in *Roseburia hominis* in the control group during pregnancy, while there is a slight increase (although it is not significant) in the MedD group.

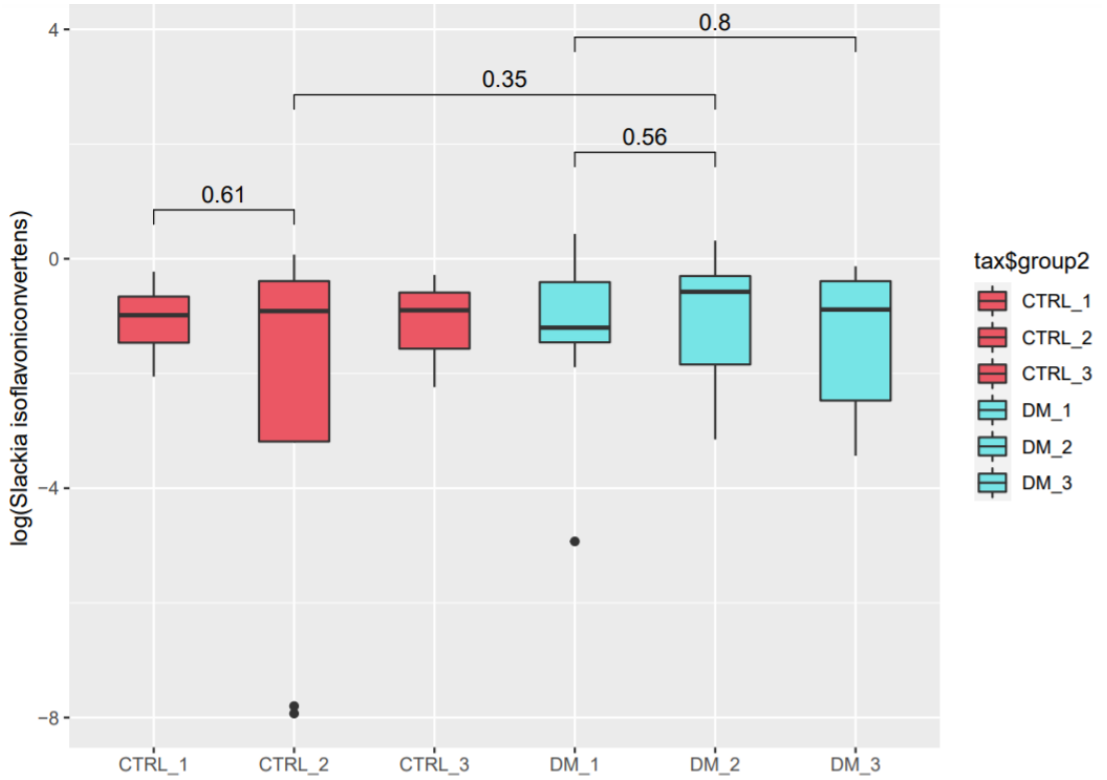

Figure 13: Box plot showing the abundance of *Slackia isoflavoniconvertens* for Mediterranean diet and control group.

There is no significant changes in *Slackia isoflavoniconvertens* in both the control and MedD groups during pregnancy.

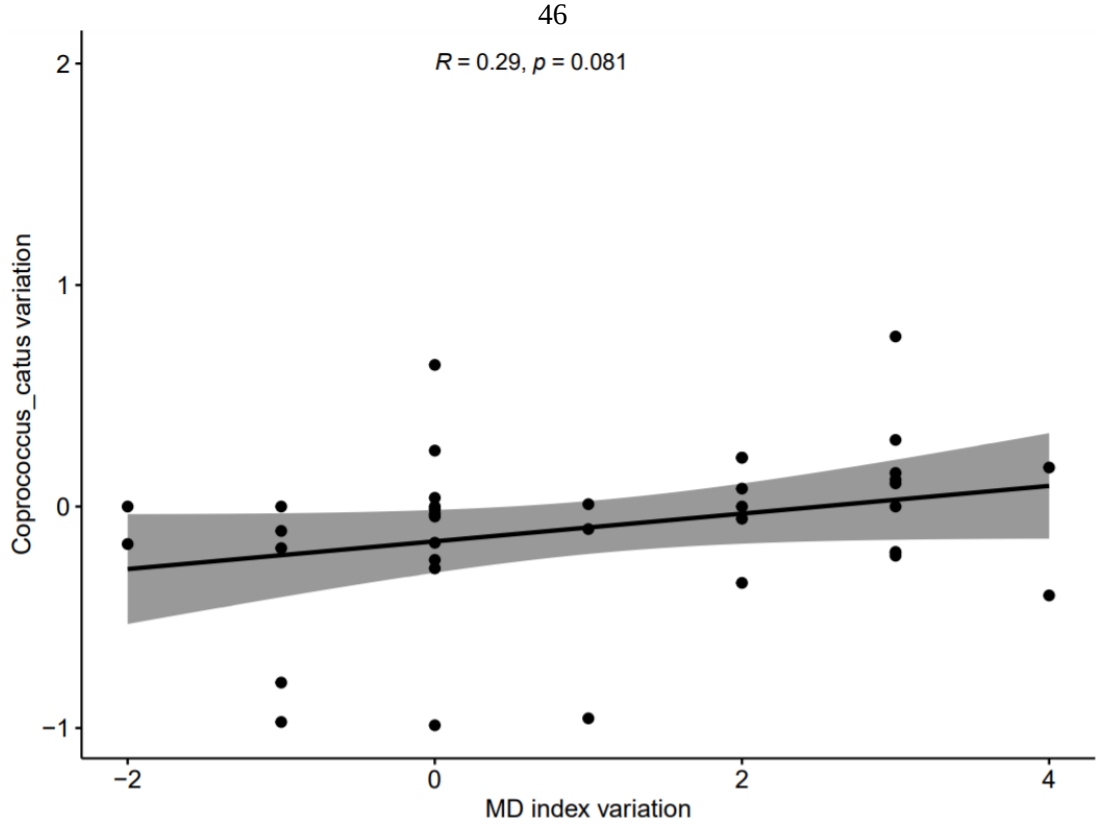

Figure 14: Spearman's correlation between the variation of MD index and *Coprococcus catus* abundance.

We explored if the variation (from the 1st to the 2nd trimester) in the Med-diet adherence index was correlated with the variation in the abundance of specific bacterial taxa. The variation in *Coprococcus catus* abundance was slightly positively correlated with the variation in the MD index (P-value=0.081, R=0.29).

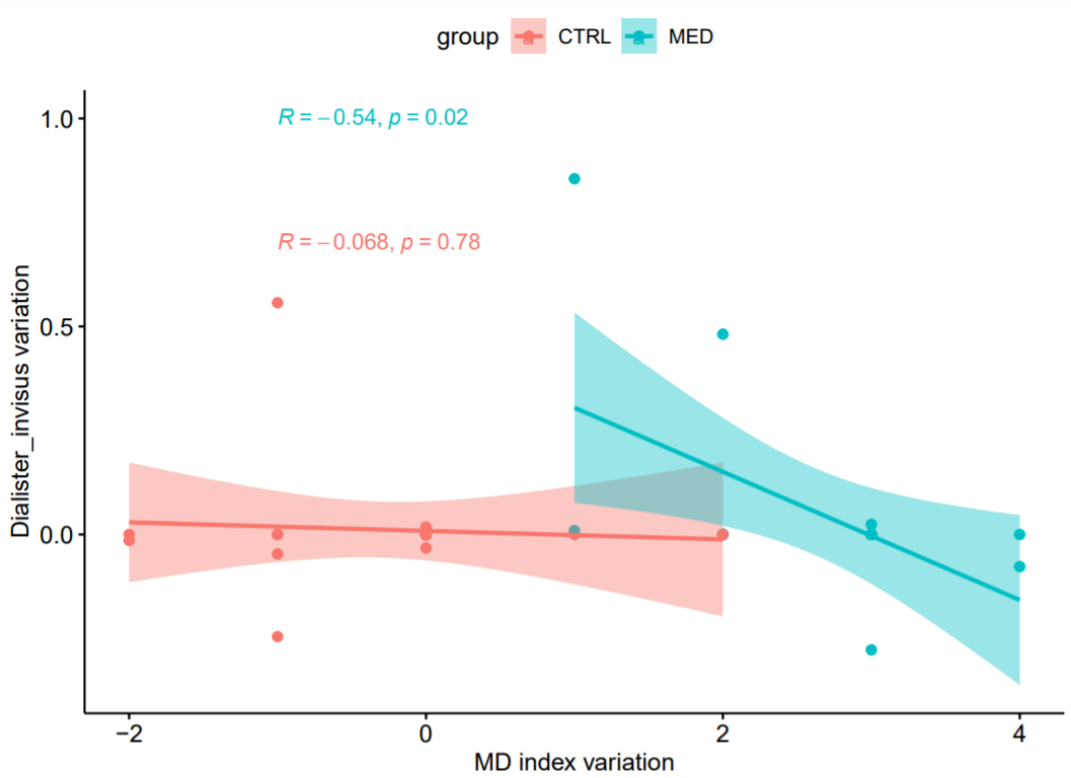

Figure 15: Spearman's correlation between the variation of MD index and *Dialister invisus* abundance.

There is no significant correlation (P-value=0.78) between the MD index and *Dialister invisus* in the control group, while there is a significantly negative correlation (P-value=0.02, R=-0.54) between MD index variation and *Dialister invisus* in MedD-group. Therefore, subjects with a higher increase in MD index at the 2nd trimester showed a decrease in *Dialister invisus.*

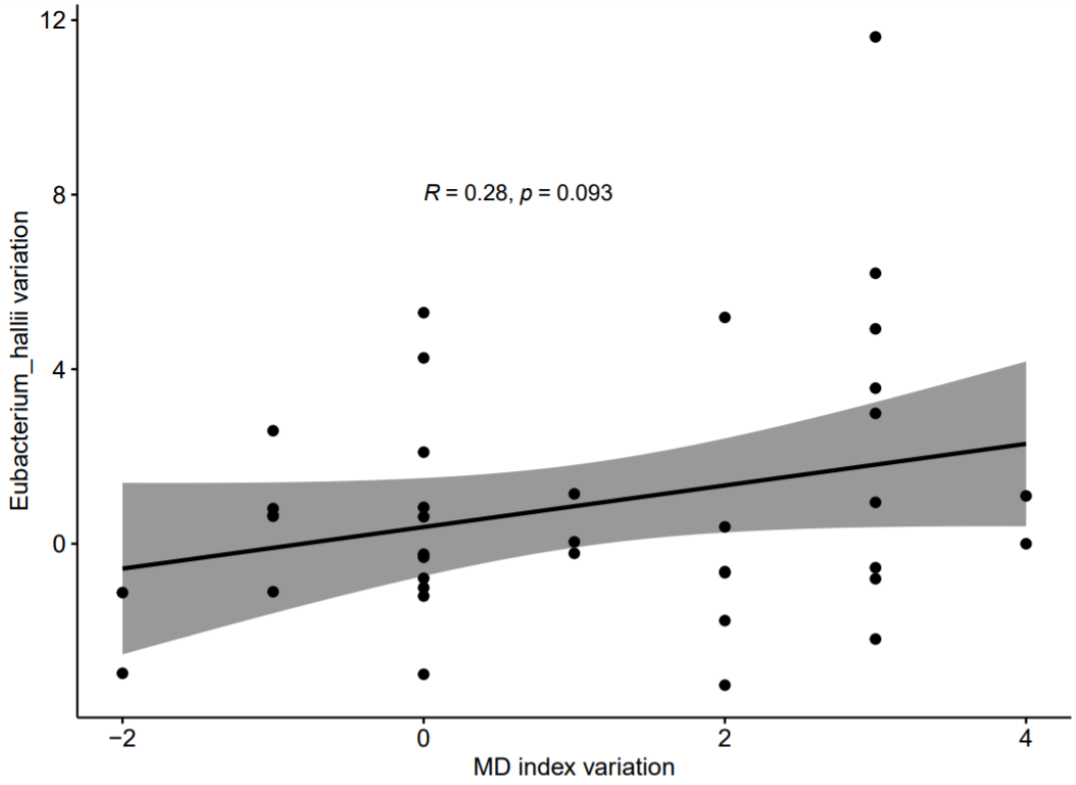

Figure 16: Spearman's correlation between the variation of MD index and *Eubacterium hallii* abundance.

There is a positive correlation (R=0.28) between the MD index and *Eubacterium hallii* abundance, although it failed to reach the significance (P-value=0.093).

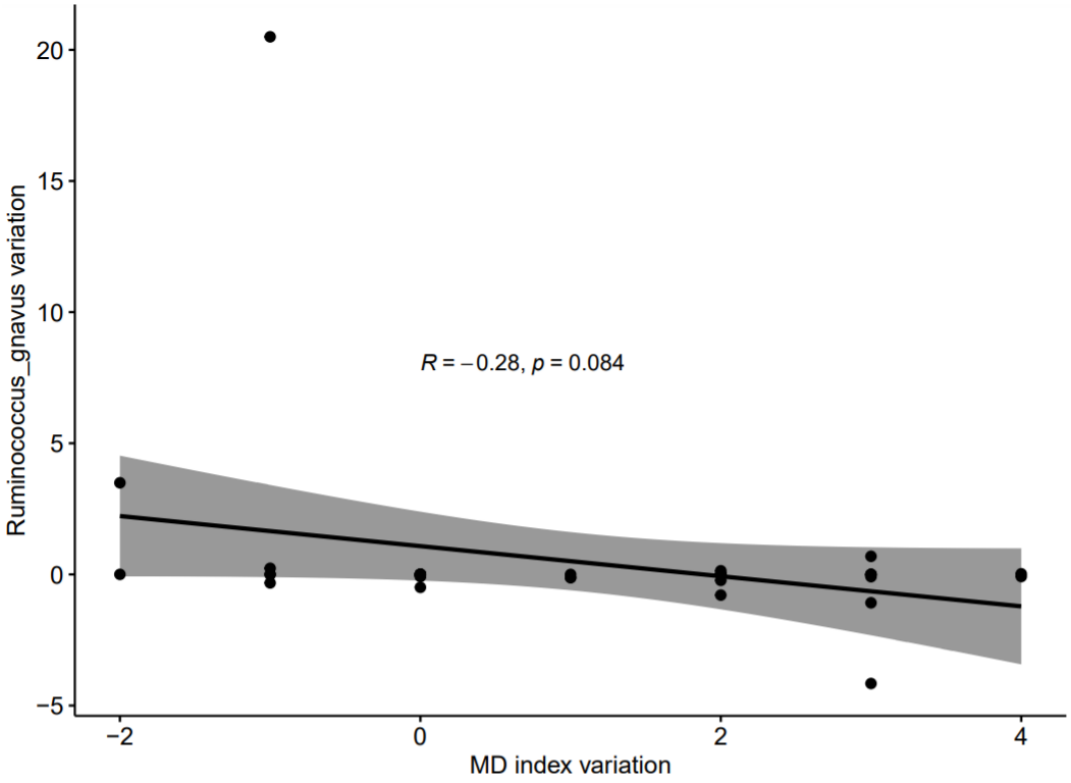

Figure 17: Spearman's correlation between the variation of MD index and *Ruminococcus gnavus* abundance.

There is a negative correlation (R=-0.28) between the MD index and *Ruminococcus gnavus*, although it failed to reach the significance (P-value=0.084).

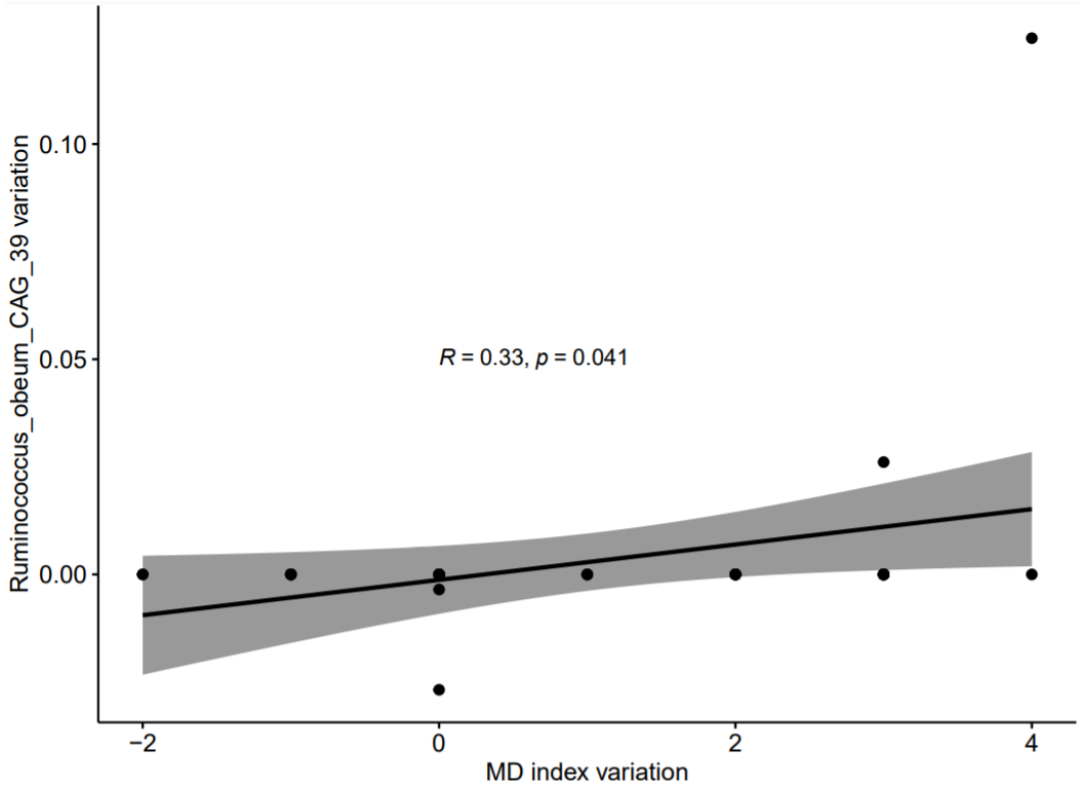

Figure 18: Spearman's correlation between the variation of MD index variation and *Ruminococcus obeum* abundance.

There is a significantly positive correlation (R=0.33) between the MD index and *Ruminococcus obeum* abundance.

4.2. Discussion

This study clearly shows that increasing the adherence to the Mediterranean dietary pattern, might help in modulating the gut microbiome during pregnancy, boosting the abundance of beneficial taxa.

Gut microbial taxonomic and functional composition in an MD intervention detects that the overall microbial richness was maintained, which is consistent with current studies that demonstrate similar trends after higher consumption of whole grains (Roager et al., 2019; Haro et al., 2016) However, we recognized that MD dynamically modifies the gut microbiome composition and that microbial differences are proportional to the increase in MD adherence index.

Each member in the MedD group followed a diet designed to increase the compliance to a typical MD pattern.

The MD intervention protocol determined a decline in *Ruminococcus gnavus* and *Actinomyces naeslundii*. These species has been demonstrated as pro-inflammatory due to the secretion of a polysaccharide that induces tumor necrosis factor-alpha in dendritic cells (Meslier et al., 2020). We observed there is a reduction in the abundance of *R. gnavus* but not significant, while *Actinomyces naeslundii* significantly decreases.

Ruminococcus genus has been reported to be enriched in individuals under a diet rich in plant polysaccharides; in addition, they are cellulolytic bacteria that utilizes cellulose and hemicellulose from plant material.

Walker et al. (2008) also showed that *Ruminococcus* species were also strongly associated with as insoluble dietary fibers present in the human colon compared to *Bacteroides*. However, our results supported the findings that difference at species level exists: while *R. bromii* and *R. obeum* can be considered as beneficial microbes, associated with fibre degradation and the production of health-promoting metabolites, such as short-chain fatty acids, other *Ruminococcus* species, e.g. *R. torques* and *R. gnavus*, may explicate an opposite effect. Moreover, our data showed the presence of *Faecalibacterium prausnitzii* in higher relative abundance in Med group. *Faecalibacterium prausnitzii* is a well-known butyrate producer, which has anti-inflammatory properties (García-Mantrana et al., 2020, Walker et al., 2008).

Many of these bacterial taxa are involved in butyrate production. It is known that fiber intake leads to increased production of short-chain fatty acids (SCFA) through microbiota fermentation. Studies have shown that *Lachnospira*, *Blautia*, *Coprococcus*, and *Bifidobacterium*, through cross-feeding interactions, consume undigestible plants polysaccharides to produce SCFA with benefits on human health (García-Mantrana et al., 2020). While in our study appeared that the *Coprococcus catus* positively correlated to the MedD index (positively increased with an increase in the adherence of MedD index), other studies conducted on MedDiet adherence reported that there is a higher abundance of *Coprococcus* and *Roseburia* (all considered as SCFA producers of the *Lachnospiraceae* family) (García-Mantrana et al., 2020).

Long-term commitment to MedD demonstrated a higher *Roseburia* sp. abundance. Notably, *Roseburia* is an important butyrate-producing genus (SCFA producer) that confer anti-inflammatory effects (Delgado-Lista et al., 2016).

We also reported an increase in *Eubacterium hallii,* SCFA producer, with higher adherence to MedD. Muralidharan et al. (2021) reported a reduction in *Eubacterium hallii* in an elderly population consuming a low calories Mediterranean diet (Muralidharan et al., 2021).

In our study, the *Eggerthellaceae* family remained constants (no significant changes). In contrast, Meslier et al. (2020) reported that the increase was consistently related to degrees of urolithin producers in microbiota, including, amongst others, individuals of the *Eggerthellaceae* family, and with consumption of nuts that have been the only dietary source of ellagitannins significantly elevated in the MedD group (Meslier et al., 2020).

Slackia isoflavoniconvertens is reported as a polyphenol degrader, with the production of urolithin. Polyphenols, and in particular the ellagitannins class, are present in red fruits and nuts, that can be considered as a typical food in MedD. Urolithins have anti-inflammatory, anticancer, and antioxidant properties. Meslier et al. (2020) reported that the increase in Egghertellaceae an Criobacteriaceae was consistently related to concentration of urolithin in an obese population consumina a MedD for 2 months (Meslier et al., 2020).

In our study, the Eggerthellaceae family remained constants (no significant changes). In contrast, Meslier et al. (2020) reported that the increase was consistently related to degrees of urolithin producers in microbiota, including, amongst others, individuals of the Eggerthellaceae family, and with consumption of nuts that have been the only dietary source of ellagitannins significantly elevated in the MedD group (Meslier et al., 2020).

Chapter Five
Conclusion

5.1 Conclusion

Increasing the adherence to the MedD in pregnant women showed a good potential for the manipulation of the gut microbiome during pregnancy, possibly improving women and infant health. Since the contact with the mom’s gut microbiome can be considered as the first microbiome inoculation in newborns (at least in those born through vaginal delivery), future studies might include also the monitoring of the effect of the dietary intervention during pregnancy in the microbiome development in newborns.

References

- Amati, F., Hassounah, S., & Swaka, A. (2019). The Impact of Mediterranean Dietary Patterns During Pregnancy on Maternal and Offspring Health. *Nutrients*, *11*(5), 1098. https://doi.org/10.3390/nu11051098

- Agnoli, C., Krogh, V., Grioni, S., Sieri, S., Palli, D., Masala, G., Sacerdote, C., Vineis, P., Tumino, R., Frasca, G., Pala, V., Berrino, F., Chiodini, P., Mattiello, A., & Panico, S. (2011). A Priori–Defined Dietary Patterns Are Associated with Reduced Risk of Stroke in a Large Italian Cohort. *The Journal of Nutrition*, *141*(8), 1552–1558. https://doi.org/10.3945/jn.111.140061

- Arrieta, M.-C., Stiemsma, L. T., Amenyogbe, N., Brown, E. M., & Finlay, B. (2014). The Intestinal Microbiome in Early Life: Health and Disease. *Frontiers in Immunology*, *5*. https://doi.org/10.3389/fimmu.2014.00427

- Bäckhed, F., Roswall, J., Peng, Y., Feng, Q., Jia, H., Kovatcheva-Datchary, P., Li, Y., Xia, Y., Xie, H., Zhong, H., Khan, M. T., Zhang, J., Li, J., Xiao, L., Al-Aama, J., Zhang, D., Lee, Y. S., Kotowska, D., Colding, C., … Wang, J. (2015). Dynamics and Stabilization of the Human Gut Microbiome during the First Year of Life. Cell Host & Microbe, 17(6), 852. https://doi.org/10.1016/j.chom.2015.05.012

- Berg, G., Rybakova, D., Fischer, D., Cernava, T., Vergès, M.-C. C., Charles, T., Chen, X., Cocolin, L., Eversole, K., Corral, G. H., Kazou, M., Kinkel, L., Lange, L., Lima, N., Loy, A., Macklin, J. A., Maguin, E., Mauchline, T., McClure, R., Schloter, M. (2020). Microbiome definition re-visited: old concepts and new challenges. *Microbiome*, *8*(1). https://doi.org/10.1186/s40168-020-00875-0

- Berman, L., & Moss, R. L. (2011). Necrotizing enterocolitis: An update. Seminars in Fetal and Neonatal Medicine, 16(3), 145–150. https://doi.org/10.1016/j.siny.2011.02.002

- Bharadia, L., Agrawal, N., & Joshi, N. (2020). Development and Functions of the Infant Gut Microflora: Western vs. Indian Infants. International Journal of Pediatrics, 2020, 1–10. https://doi.org/10.1155/2020/7586264

- Bik, E. M., Eckburg, P. B., Gill, S. R., Nelson, K. E., Purdom, E. A., Francois, F., Perez-Perez, G., Blaser, M. J., & Relman, D. A. (2006). Molecular analysis of the bacterial microbiota in the human stomach. *Proceedings of the National Academy of Sciences*, *103*(3), 732–737. https://doi.org/10.1073/pnas.0506655103

- Booijink, C. C., El-Aidy, S., Rajilić-Stojanović, M., Heilig, H. G., Troost, F. J., Smidt, H., Kleerebezem, M., De Vos, W. M., & Zoetendal, E. G. (2010). High temporal and inter-individual variation detected in the human ileal microbiota. *Environmental Microbiology*, *12*(12), 3213–3227. https://doi.org/10.1111/j.1462-2920.2010.02294.x

- Carding, S., Verbeke, K., Vipond, D. T., Corfe, B. M., & Owen, L. J. (2015). Dysbiosis of the gut microbiota in disease. *Microbial Ecology in Health & Disease*, *26*. https://doi.org/10.3402/mehd.v26.26191

- Chatzi, L., Rifas-Shiman, S. L., Georgiou, V., Joung, K. E., Koinaki, S., Chalkiadaki, G., Margioris, A., Sarri, K., Vassilaki, M., Vafeiadi, M., Kogevinas, M., Mantzoros, C., Gillman, M. W., & Oken, E. (2017). Adherence to the Mediterranean diet during pregnancy and offspring adiposity and cardiometabolic traits in childhood. *Pediatric Obesity*, *12*, 47–56. https://doi.org/10.1111/ijpo.12191

- Chen, C.-L., Wong, M.-W., Yi, C.-H., Liu, T.-T., Lei, W.-Y., Hung, J.-S., Lin, C.-L., & Lin, S.-Z. (2018). Impact of vegan diets on gut microbiota: An update on the clinical implications. *Tzu Chi Medical Journal*, *30*(4), 200. https://doi.org/10.4103/tcmj.tcmj_21_18

- Chen, Y., Ji, F., Guo, J., Shi, D., Fang, D., & Li, L. (2016). Dysbiosis of small intestinal microbiota in liver cirrhosis and its association with etiology. Scientific Reports, 6(1). https://doi.org/10.1038/srep34055

- Chiavaroli, L., Viguiliouk, E., Nishi, S., Blanco Mejia, S., Rahelić, D., Kahleová, H., Salas-Salvadó, J., Kendall, C., & Sievenpiper, J. (2019). DASH Dietary Pattern and Cardiometabolic Outcomes: An Umbrella Review of Systematic Reviews and Meta-Analyses. *Nutrients*, *11*(2), 338. https://doi.org/10.3390/nu11020338

- Chong, C., Bloomfield, F., & O'Sullivan, J. (2018). Factors Affecting Gastrointestinal Microbiome Development in Neonates. Nutrients, 10(3), 274. https://doi.org/10.3390/nu10030274

- Clemente, J. C., Pehrsson, E. C., Blaser, M. J., Sandhu, K., Gao, Z., Wang, B., Magris, M., Hidalgo, G., Contreras, M., Noya-Alarcón, Ó., Lander, O., McDonald, J., Cox, M., Walter, J., Oh, P. L., Ruiz, J. F., Rodriguez, S., Shen, N., Song, S. J., … Dominguez-Bello, M. G. (2015). The microbiome of uncontacted Amerindians. *Science Advances*, *1*(3). https://doi.org/10.1126/sciadv.1500183

- David, L. A., Maurice, C. F., Carmody, R. N., Gootenberg, D. B., Button, J. E., Wolfe, B. E., Ling, A. V., Devlin, A. S., Varma, Y., Fischbach, M. A., Biddinger, S. B., Dutton, R. J., & Turnbaugh, P. J. (2013). Diet rapidly and reproducibly alters the human gut microbiome. *Nature*, *505*(7484), 559–563. https://doi.org/10.1038/nature12820

- *De Filippis, F., & Ercolini, D. (2018).* Microbiome and Diet. *The Gut Microbiome in Health and Disease*, 79–88. https://doi.org/10.1007/978-3-319-90545-7_6

- *DeGruttola, A. K., Low, D., Mizoguchi, A., & Mizoguchi, E. (2016).* Current Understanding of Dysbiosis in Disease in Human and Animal Models. *Inflammatory Bowel Diseases*, *22*(5), 1137–1150. https://doi.org/10.1097/mib.0000000000000750

- *Delgado, S., Cabrera-Rubio, R., Mira, A., Suárez, A., & Mayo, B. (2013).* Microbiological Survey of the Human Gastric Ecosystem Using Culturing and Pyrosequencing Methods. *Microbial Ecology*, *65*(3), 763–772. https://doi.org/10.1007/s00248-013-0192-5

- Delgado-Lista, J., Perez-Martinez, P., Garcia-Rios, A., Alcala-Diaz, J. F., Perez-Caballero, A. I., Gomez-Delgado, F., Fuentes, F., Quintana-Navarro, G., Lopez-Segura, F., Ortiz-Morales, A. M., Delgado-Casado, N., Yubero-Serrano, E. M., Camargo, A., Marin, C., Rodriguez-Cantalejo, F., Gomez-Luna, P., Ordovas, J. M., Lopez-Miranda, J., & Perez-Jimenez, F. (2016). CORonary Diet Intervention with Olive oil and cardiovascular PREVention study (the CORDIOPREV study): Rationale, methods, and baseline characteristics. *American Heart Journal*, *177*, 42–50. https://doi.org/10.1016/j.ahj.2016.04.011

- Dieterich, W., Schink, M., & Zopf, Y. (2018). Microbiota in the Gastrointestinal Tract. *Medical Sciences*, *6*(4), 116. https://doi.org/10.3390/medsci6040116

- Dogra, S., Sakwinska, O., Soh, S. E., Ngom-Bru, C., Bruck, W. M., Berger, B., Brussow, H., Lee, Y. S., Yap, F., Chong, Y. S., et al (2015). Dynamics of infant gut microbiota are influenced by delivery mode and gestational duration and are associated with subsequent adiposity. MBio, 6, e02419–e02414.

- Dominguez-Bello, M. G., De Jesus-Laboy, K. M., Shen, N., Cox, L. M., Amir, A., Gonzalez, A., Bokulich, N. A., Song, S. J., Hoashi, M., Rivera-Vinas, J. I., et al. (2016). Partial restoration of the microbiota of cesarean-born infants via vaginal microbial transfer. Nature Medicine, 22, 250–253

- Egan, C. E., Sodhi, C. P., Good, M., Lin, J., Jia, H., Yamaguchi, Y., Lu, P., Ma, C., Branca, M. F., Weyandt, S., Fulton, W. B., Niño, D. F., Prindle, T., Ozolek, J. A., & Hackam, D. J. (2015). Toll-like receptor 4–mediated lymphocyte influx induces neonatal necrotizing enterocolitis. *Journal of Clinical Investigation*, *126*(2), 495–508. https://doi.org/10.1172/jci83356

- *Ferrocino, I., Ponzo, V., Gambino, R., Zarovska, A., Leone, F., Monzeglio, C., Goitre, I., Rosato, R., Romano, A., Grassi, G., Broglio, F., Cassader, M., Cocolin, L., & Bo, S. (2018).* Changes in the gut microbiota composition during pregnancy in patients with gestational diabetes mellitus (GDM). *Scientific Reports*, *8*(1). https://doi.org/10.1038/s41598-018-30735-9

- *Garcia-Mantrana, I., Selma-Royo, M., Alcantara, C., & Collado, M. C. (2018).* Shifts on Gut Microbiota Associated to Mediterranean Diet Adherence and Specific Dietary Intakes on General Adult Population. *Frontiers in Microbiology*, *9*. https://doi.org/10.3389/fmicb.2018.00890

- García-Mantrana, I., Selma-Royo, M., González, S., Parra-Llorca, A., Martínez-Costa, C., & Collado, M. C. (2020). Distinct maternal

microbiota clusters are associated with diet during pregnancy: impact on neonatal microbiota and infant growth during the first 18 months of life. *Gut Microbes*, *11*(4), 962–978. https://doi.org/10.1080/19490976.2020.1730294

- Grosicki, G. J., Fielding, R. A., & Lustgarten, M. S. (2017). Gut Microbiota Contribute to Age-Related Changes in Skeletal Muscle Size, Composition, and Function: Biological Basis for a Gut-Muscle Axis. *Calcified Tissue International*, *102*(4), 433–442. https://doi.org/10.1007/s00223-017-0345-5

- Hackam, D. J., Afrazi, A., Good, M., & Sodhi, C. P. (2013). Innate Immune Signaling in the Pathogenesis of Necrotizing Enterocolitis. *Clinical and Developmental Immunology*, *2013*, 1–10. https://doi.org/10.1155/2013/475415

- Haller, D. (2018). The gut microbiome in health and disease. Springer.

- Haro C, Montes-Borrego M, Rangel-Zúñiga OA, Alcalá-Díaz JF, Gómez-Delgado F, Pérez-Martínez P, Delgado-Lista J, Quintana-Navarro GM, Tinahones FJ, Landa BB. 2016. Two healthy diets modulate gut microbial community improving insulin sensitivity in a human obese population. J Clin Endocrinol 101:233–242.*a*

- Henderickx, J. G., Zwittink, R. D., van Lingen, R. A., Knol, J., & Belzer, C. (2019). The Preterm Gut Microbiota: An Inconspicuous Challenge in Nutritional Neonatal Care. *Frontiers in Cellular and Infection Microbiology*, *9*. https://doi.org/10.3389/fcimb.2019.00085

- Hesla, H. M., Stenius, F., Jaderlund, L., Nelson, R., Engstrand, L., Alm, J., & Dicksved, J. (2014). Impact of lifestyle on the gut microbiota of healthy infants and their mothers-the ALADDIN birth cohort. FEMS Microbiology Ecology, 90, 791–801

- Hill, C. J., Lynch, D. B., Murphy, K., Ulaszewska, M., Jeffery, I. B., O'Shea, C. A., Watkins, C., Dempsey, E., Mattivi, F., Tuohy, K., Ross, R. P., Ryan, C. A., O'Toole, P. W., & Stanton, C. (2017). Erratum to: Evolution of gut microbiota composition from birth to 24 weeks in the INFANTMET Cohort. *Microbiome*, *5*(1). https://doi.org/10.1186/s40168-017-0240-3

- Hornef, M. (2018). Microbiome and Early Life. *The Gut Microbiome in Health and Disease*, 31–47. https://doi.org/10.1007/978-3-319-90545-7_3

- Hoyles, L., Fernández-Real, J.-M., Federici, M., Serino, M., Abbott, J., Charpentier, J., Heymes, C., Luque, J. L., Anthony, E., Barton, R. H., Chilloux, J., Myridakis, A., Martinez-Gili, L., Moreno-Navarrete, J. M., Benhamed, F., Azalbert, V., Blasco-Baque, V., Puig, J., Xifra, G., Dumas, M.-E. (2018). Molecular phenomics and metagenomics of

hepatic steatosis in non-diabetic obese women. *Nature Medicine*, *24*(7), 1070–1080. https://doi.org/10.1038/s41591-018-0061-3

- Humphreys, C. (2020). Intestinal Permeability. *Textbook of Natural Medicine*. https://doi.org/10.1016/b978-0-323-43044-9.00019-4

- Jakobsson, H. E., Abrahamsson, T. R., Jenmalm, M. C., Harris, K., Quince, C., Jernberg, C., Bjorksten, B., Engstrand, L., & Andersson, A. F. (2014). Decreased gut microbiota diversity, delayed Bacteroidetes colonisation and reduced Th1 responses in infants delivered by caesarean section. Gut, 63, 559–566.

- Jananee Muralidharan, Isabel Moreno-Indias, Mónica Bulló, Jesús Vioque Lopez, Dolores Corella, Olga Castañer, Josep Vidal, Alessandro Atzeni, Jose Carlos Fernandez-García, Laura Torres-Collado, Rebeca Fernández-Carrión, Monsterrat Fito, Romina Olbeyra, Ana Maria Gomez-Perez, Serena Galiè, Maria Rosa Bernal-López, Miguel Angel Martinez-Gonzalez, Jordi Salas-Salvadó, Francisco Jose Tinahones, Effect on gut microbiota of a 1-y lifestyle intervention with Mediterranean diet compared with energy-reduced Mediterranean diet and physical activity promotion: PREDIMED-Plus Study, The American Journal of Clinical Nutrition, 2021;, nqab150, https://doi.org/10.1093/ajcn/nqab150

- Jandhyala, S. M. (2015). Role of the normal gut microbiota. *World Journal of Gastroenterology*, *21*(29), 8787. https://doi.org/10.3748/wjg.v21.i29.8787

- Kelly, J. T., Palmer, S. C., Wai, S. N., Ruospo, M., Carrero, J.-J., Campbell, K. L., & Strippoli, G. F. (2016). Healthy Dietary Patterns and Risk of Mortality and ESRD in CKD: A Meta-Analysis of Cohort Studies. *Clinical Journal of the American Society of Nephrology*, *12*(2), 272–279. https://doi.org/10.2215/cjn.06190616

- Kotloff, K. L., Nataro, J. P., Blackwelder, W. C., Nasrin, D., Farag, T. H., Panchalingam, S., Wu, Y., Sow, S. O., Sur, D., Breiman, R. F., Faruque, A. S. G., Zaidi, A. K. M., Saha, D., Alonso, P. L., Tamboura, B., Sanogo, D., Onwuchekwa, U., Manna, B., Ramamurthy, T., … Levine, M. M. (2013). Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. *The Lancet*, *382*(9888), 209–222. https://doi.org/10.1016/s0140-6736(13)60844-2

- Kumbhare, S. V., Patangia, D. V., Patil, R. H., Shouche, Y. S., & Patil, N. P. (2019). Factors influencing the gut microbiome in children: from infancy to childhood. *Journal of Biosciences*, *44*(2). https://doi.org/10.1007/s12038-019-9860-z

- Leshem, A., Segal, E., & Elinav, E. (2020). The Gut Microbiome and Individual-Specific Responses to Diet. *MSystems*, *5*(5). https://doi.org/10.1128/msystems.00665-20

- *Li, N., Zuo, B., Huang, S., Zeng, B., Han, D., Li, T., Liu, T., Wu, Z., Wei, H., Zhao, J., & Wang, J. (2020).* Spatial heterogeneity of bacterial colonization across different gut segments following inter-species microbiota transplantation. *Microbiome*, *8*(1). https://doi.org/10.1186/s40168-020-00917-7

- Louis, P., Hold, G. L., & Flint, H. J. (2014). The gut microbiota, bacterial metabolites and colorectal cancer. *Nature Reviews Microbiology*, *12*(10), 661–672. https://doi.org/10.1038/nrmicro3344

- Lu, C.-Y., & Ni, Y.-H. (2015). Gut microbiota and the development of pediatric diseases. *Journal of Gastroenterology*, *50*(7), 720–726. https://doi.org/10.1007/s00535-015-1082-z

- Mailhe, M., Ricaboni, D., Vitton, V., Gonzalez, J.-M., Bachar, D., Dubourg, G., Cadoret, F., Robert, C., Delerce, J., Levasseur, A., Fournier, P.-E., Angelakis, E., Lagier, J.-C., & Raoult, D. (2018). Repertoire of the gut microbiota from stomach to colon using culturomics and next-generation sequencing. *BMC Microbiology*, *18*(1). https://doi.org/10.1186/s12866-018-1304-7

- Marques, T. M., Wall, R., Ross, R. P., Fitzgerald, G. F., Ryan, C. A., & Stanton, C. (2010). Programming infant gut microbiota: influence of dietary and environmental factors. *Current Opinion in Biotechnology*, *21*(2), 149–156. https://doi.org/10.1016/j.copbio.2010.03.020

- Matsuki, T., Yahagi, K., Mori, H., Matsumoto, H., Hara, T., Tajima, S., Ogawa, E., Kodama, H., Yamamoto, K., Yamada, T., Matsumoto, S., & Kurokawa, K. (2016). A key genetic factor for fucosyllactose utilization affects infant gut microbiota development. *Nature Communications*, *7*(1). https://doi.org/10.1038/ncomms11939

- McKnite, A. M., Perez-Munoz, M. E., Lu, L., Williams, E. G., Brewer, S., Andreux, P. A., Bastiaansen, J. W., Wang, X., Kachman, S. D., Auwerx, J., Williams, R. W., Benson, A. K., Peterson, D. A., & Ciobanu, D. C. (2012). Murine Gut Microbiota Is Defined by Host Genetics and Modulates Variation of Metabolic Traits. *PLoS ONE*, *7*(6). https://doi.org/10.1371/journal.pone.0039191

- *Merra, G., Noce, A., Marrone, G., Cintoni, M., Tarsitano, M. G., Capacci, A., & De Lorenzo, A. (2020).* Influence of Mediterranean Diet on Human Gut Microbiota. *Nutrients*, *13*(1), 7. https://doi.org/10.3390/nu13010007

- Meslier, V., Laiola, M., Roager, H. M., De Filippis, F., Roume, H., Quinquis, B., Giacco, R., Mennella, I., Ferracane, R., Pons, N., Pasolli, E., Rivellese, A., Dragsted, L. O., Vitaglione, P., Ehrlich, S. D., & Ercolini, D. (2020). Mediterranean diet intervention in overweight and obese subjects lowers plasma cholesterol and causes changes in the gut microbiome and metabolome independently of energy intake. *Gut*, *69*(7), 1258–1268. https://doi.org/10.1136/gutjnl-2019-320438

- Moles, L., & Otaegui, D. (2020). The Impact of Diet on Microbiota Evolution and Human Health. Is Diet an Adequate Tool for Microbiota Modulation? *Nutrients*, *12*(6), 1654. https://doi.org/10.3390/nu12061654

- Muralidharan, J., Indias, I., Bulló, M., Lopez, J., Corella, D., Castañer, O., Vidal, J., Atzeni, A., García, J., et al. (2021). Effect on gut microbiota of a 1-y lifestyle intervention with Mediterranean diet compared with energy-reduced Mediterranean diet and physical activity promotion: PREDIMED-Plus Study. The American Journal of Clinical Nutrition, nqab150, https://doi.org/10.1093/ajcn/nqab150

- Nardone, G., & Compare, D. (2015). The human gastric microbiota: Is it time to rethink the pathogenesis of stomach diseases? *United European Gastroenterology Journal*, *3*(3), 255–260. https://doi.org/10.1177/2050640614566846

- Nuriel-Ohayon, M., Neuman, H., & Koren, O. (2016). Microbial Changes during Pregnancy, Birth, and Infancy. *Frontiers in Microbiology*, *7*. https://doi.org/10.3389/fmicb.2016.01031

- O'Keefe, S. J. (2016). Diet, microorganisms and their metabolites, and colon cancer. *Nature Reviews Gastroenterology & Hepatology*, *13*(12), 691–706. https://doi.org/10.1038/nrgastro.2016.165

- Pedersen, H. K., Gudmundsdottir, V., Nielsen, H. B., Hyotylainen, T., Nielsen, T., Jensen, B. A., Forslund, K., Hildebrand, F., Prifti, E., Falony, G., Le Chatelier, E., Levenez, F., Doré, J., Mattila, I., Plichta, D. R., Pöhö, P., Hellgren, L. I., Arumugam, M., Sunagawa, S., … Pedersen, O. (2016). Human gut microbes impact host serum metabolome and insulin sensitivity. *Nature*, *535*(7612), 376–381. https://doi.org/10.1038/nature18646

- Penders, J., Gerhold, K., Stobberingh, E. E., Thijs, C., Zimmermann, K., Lau, S., & Hamelmann, E. (2013). Establishment of the intestinal microbiota and its role for atopic dermatitis in early childhood. The Journal of Allergy and Clinical Immunology, 132, 601–607

- Roager, H. M., & Dragsted, L. O. (2019). Diet-derived microbial metabolites in health and disease. *Nutrition Bulletin*, *44*(3), 216–227. https://doi.org/10.1111/nbu.12396

- Roager HM, Vogt JK, Kristensen M, Hansen LBS, Ibrügger S, Mærkedahl RB, Bahl MI, Lind MV, Nielsen RL, Frøkiær H. 2019. Whole grain-rich diet reduces body weight and systemic low-grade inflammation without inducing major changes of the gut microbiome: a randomised cross-over trial. Gut 68:83–93.

- Rodríguez, J. M., Murphy, K., Stanton, C., Ross, R. P., Kober, O. I., Juge, N., Avershina, E., Rudi, K., Narbad, A., Jenmalm, M. C., Marchesi, J. R., & Collado, M. C. (2015). The composition of the gut microbiota throughout life, with an emphasis on early life. *Microbial*

Ecology in Health & Disease, *26*. https://doi.org/10.3402/mehd.v26.26050

- Roze, J. C., Ancel, P. Y., Lepage, P., Martin-Marchand, L., Al Nabhani, Z., Delannoy, J., Picaud, J. C., Lapillonne, A., Aires, J., Durox, M., et al. (2017). Nutritional strategies and gut microbiota composition as risk factors for necrotizing enterocolitis in verypreterm infants. The American Journal of Clinical Nutrition, 106, 821–830.

- Sakurama, H., Kiyohara, M., Wada, J., Honda, Y., Yamaguchi, M., Fukiya, S., Yokota, A., Ashida, H., Kumagai, H., Kitaoka, M., Yamamoto, K., & Katayama, T. (2013). Lacto-N-biosidase Encoded by a Novel Gene of Bifidobacterium longum Subspecies longum Shows Unique Substrate Specificity and Requires a Designated Chaperone for Its Active Expression. *Journal of Biological Chemistry*, *288*(35), 25194–25206. https://doi.org/10.1074/jbc.m113.484733

- *Sarkar, A., Yoo, J. Y., Valeria Ozorio Dutra, S., Morgan, K. H., & Groer, M. (2020).* The Association Between Early-Life Gut Microbiota and Long-Term Health and Diseases. https://doi.org/10.20944/preprints202011.0683.v1

- Selma-Royo, M., García-Mantrana, I., Calatayud, M., Parra-Llorca, A., Martínez-Costa, C., & Collado, M. C. (2020). Maternal diet during pregnancy and intestinal markers are associated with early gut microbiota. *European Journal of Nutrition*, *60*(3), 1429–1442. https://doi.org/10.1007/s00394-020-02337-7

- Sender, R., Fuchs, S., & Milo, R. (2016). Revised estimates for the number of human and bacteria cells in the body. https://doi.org/10.1101/036103

- Tanaka, M., & Nakayama, J. (2017). Development of the gut microbiota in infancy and its impact on health in later life. *Allergology International*, *66*(4), 515–522. https://doi.org/10.1016/j.alit.2017.07.010

- Walker, A. W., Duncan, S. H., Harmsen, H. J., Holtrop, G., Welling, G. W., & Flint, H. J. (2008). The species composition of the human intestinal microbiota differs between particle-associated and liquid phase communities. *Environmental Microbiology*, *10*(12), 3275–3283. https://doi.org/10.1111/j.1462-2920.2008.01717.x

- Wall, R., Ross, R. P., Ryan, C. A., Hussey, S., Murphy, B., Fitzgerald, G. F., & Stanton, C. (2009). Role of Gut Microbiota in Early Infant Development. *Clinical Medicine. Pediatrics*, *3*. https://doi.org/10.4137/cmped.s2008

- Vatanen, T., Kostic, A. D., d'Hennezel, E., Siljander, H., Franzosa, E. A., Yassour, M., Kolde, R., Vlamakis, H., Arthur, T. D., Hämäläinen, A.-M., Peet, A., Tillmann, V., Uibo, R., Mokurov, S., Dorshakova, N., Ilonen, J., Virtanen, S. M., Szabo, S. J., Porter, J. A., … Xavier, R. J. (2016). Variation in Microbiome LPS Immunogenicity Contributes to Autoimmunity in Humans. *Cell*, *165*(6), 1551. https://doi.org/10.1016/j.cell.2016.05.056

- *Vernocchi, P., Del Chierico, F., & Putignani, L. (2020).* Gut Microbiota Metabolism and Interaction with Food Components. *International Journal of Molecular Sciences*, *21*(10), 3688. https://doi.org/10.3390/ijms21103688

- Wang, S., Egan, M., Ryan, C. A., Boyaval, P., Dempsey, E. M., Ross, R. P., & Stanton, C. (2020). A good start in life is important—perinatal factors dictate early microbiota development and longer term maturation. *FEMS Microbiology Reviews*, *44*(6), 763–781. https://doi.org/10.1093/femsre/fuaa030

- Wang, Y., Qi, W., Song, G., Pang, S., Peng, Z., Li, Y., & Wang, P. (2020). High-Fructose Diet Increases Inflammatory Cytokines and Alters Gut Microbiota Composition in Rats. *Mediators of Inflammation*, *2020*, 1–10. https://doi.org/10.1155/2020/6672636

- Worsøe, J., Fynne, L., Gregersen, T., Schlageter, V., Christensen, L. A., Dahlerup, J. F., Rijkhoff, N. J. M., Laurberg, S., & Krogh, K. (2011). Gastric transit and small intestinal transit time and motility assessed by a magnet tracking system. *BMC Gastroenterology*, *11*(1). https://doi.org/10.1186/1471-230x-11-145

- Yang, I., Corwin, E. J., Brennan, P. A., Jordan, S., Murphy, J. R., & Dunlop, A. (2016). The Infant Microbiome. *Nursing Research*, *65*(1), 76–88. https://doi.org/10.1097/nnr.0000000000000133

- Zwittink, R. D., van Zoeren-Grobben, D., Martin, R., van Lingen, R. A., Groot Jebbink, L. J., Boeren, S., Renes, I. B., van Elburg, R. M., Belzer, C., & Knol, J. (2017). Metaproteomics reveals functional differences in intestinal microbiota development of preterm infants. *Molecular & Cellular Proteomics*, *16*(9), 1610–1620. https://doi.org/10.1074/mcp.ra117.000102

تأثير حمية البحر الابيض المتوسط على بكتيريا الامعاء للحوامل: دراسة للسيطرة على الحالات

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الملخص

الهدف:

الهدف الرئيسي من هذه الدراسة هو تقييم التأثير المحتمل للالتزام بنظام البحر الأبيض المتوسط الغذائي على تكوين ميكروبيوم الأمعاء أثناء الحمل.

طريقة البحث:

تم اجراء هذا البحث على ستة وسبعون حامل لا تعاني من اي امراض لمدة تسع اشهر(طوال فترة الحمل)، خمسة وثلاثون امرأة استهلكوا حمية البحر الابيض المتوسط وواحد واربعون امرأة استهلكوا النظام الغذائي اليومي. تمت مراقبة الالتزام الغذائي وميكروبيوم الأمعاء خلال فترة الدراسة.

النتائج:

اظهرت النتائج ان غالبية النساء الحوامل التزموا في حمية البحر الابيض المتوسط، ووجد انه هناك علاقة بين استهلاك حمية البحر الابيض المتوسط ووفرة بكتيريا الامعاء المفيدة، مثل البكتيريا المسؤلة عن الالياف وبعض الاصناف المرتبطة بتحلل البوليفينول.

الاستنتاجات والتوصيات:

يمكن ان نستنتج من النتائج انه استهلاك حمية البحر الابيض المتوسط خلال الحمل هي طريقة واعدة لتحسين ميكروبيوم الامعاء، مما قد يؤثر على تطور ميكروبيوم الأمعاء عند الأطفال حديثي الولادة.